The Ecology, Impacts, and Control of *Crassula helmsii*

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This thesis has been submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy

Bournemouth University in collaboration with the National Trust and with the Royal Society for the Protection of Birds

June 2015

Abstract

A major focus of research regarding non-native invasive plants is to understand what ecological variables (abiotic and biotic) increase or decrease the likelihood that a non-native plant species will become invasive, and to determine whether invasion has a negative impact on native plant species. *Crassula helmsii* is a plant indigenous to Australasia, which has invaded wetland habitats across much of Britain. It is a low growing, clonally spreading plant, which occupies the margins and shallows of freshwater and brackish waterbodies. *Crassula helmsii* is considered invasive due to its ability to produce vigorous aboveground growth, which in some locations spreads to form dense monospecific mats of vegetation. There are concerns that *C. helmsii* can exclude native plant species, and therefore that its invasion may have a negative impact on biodiversity. The research presented in this thesis was conducted with the aim of better understanding the mechanisms which allow *C. helmsii* to develop dense, dominating vegetative mats. This aim was addressed by investigating how *C. helmsii* abundance was related to variation in the abiotic environment, how *C. helmsii* abundance was affected by generalist herbivores, and whether *C. helmsii* abundance was related to the presence or absence of co-occurring plant competitors.

To investigate how *C. helmsii* abundance is related to the abiotic environment, firstly field data were collected of pH, nutrient levels, water depth, and shade levels along *C. helmsii* invaded margins of waterbodies. Multiple linear regression was used to address the question: How well does abiotic variation predict variation in *C. helmsii* abundance in the field? Finding that *C. helmsii* cover was significantly greater at a higher pH and at lower shade levels. Secondly *C. helmsii* was grown in mesocosms at different strengths of salinity (0, 2, 4, 8 ppt) to address the question: What is the estimated minimum salinity level for the control of *C. helmsii* in brackish water habitats? Relative growth rate decreased significantly with increasing salinity and *C. helmsii* died in the highest salinity treatments (8 ppt). These two experiments demonstrated that *C. helmsii* abundance can be affected by the abiotic environment, and specifically by pH, shade levels, and salinity. Dense, dominating mats of *C. helmsii* are more likely to occur in freshwater conditions, which have a neutral to alkaline pH, and with minimal overhead shading. More effective control against *C. helmsii* dominance may be achieved by preventing *C. helmsii* from colonising sites with such abiotic conditions, or by focusing more effort on removal of *C. helmsii* vegetation which has colonised such sites.

To investigate the effect of disturbance from generalist herbivores, a field-based experiment was set up, in which 4 m^2 fenced plots which excluded large vertebrates were erected along a *C. helmsii* invaded drawdown zone. The abundances of all plants species within the fenced plots and within adjacent unfenced plots were recorded repeatedly over a year, and compared, in order to explore the question: What is the effect of grazing on the abundance of *C. helmsii*

within a wetland plant community? *Crassula helmsii* declined in abundance in the fenced plots compared to the unfenced plots, and the abundance of co-occurring vegetation was consistently higher in the fenced plots than in the unfenced plots. *Crassula helmsii* appeared to benefit from grazing disturbance, attaining a higher abundance in the unfenced plots, where competitor plant species had been grazed out. *Crassula helmsii* could have a negative impact on native plant species diversity, if it is able to fill a niche usually occupied by native plant species of open, grazed drawdown zones (e.g. *Teucrium scordium*).

The relationship between *C. helmsii* abundance and the presence or absence of co-occurring plants was investigated in order to ascertain the effect of competition on the abundance of *C. helmsii*, and also to ascertain the effect of competition from *C. helmsii* on the abundance and diversity of co-occurring plants, therefore giving an indication of whether the spread of *C. helmsii* can have a negative impact on native species. Firstly a common garden experiment and a field based experiment were separately conducted, both assessing how well *C. helmsii* could spread into available bare ground compared to native competitors, and addressing the question: Is available bare ground an important pre-requisite to high *C. helmsii* abundance? These experiments found that *C. helmsii* can rapidly respond to available bare ground, but that simultaneous spread of native competitors might limit *C. helmsii* community dominance. The effect of *C. helmsii* on native plants was species specific; the common garden experiment showed that *C. helmsii* limited the spread of *Anagallis tenella*, but not *Hypericum elodes* or *Hydrocotyle vulgaris*. Secondly field survey data of plant species abundance and diversity were collected to explore the question: Can the invasion of *C. helmsii* negatively affect the plant community in a *Phragmites australis* dominated fen habitat? Survey data were analysed to determine whether *C. helmsii* was negatively correlated with variables of the plant community, a pattern indicating that *C. helmsii* could increase in abundance by displacing native plants. The variables 'total community abundance' and 'species diversity' were not significantly correlated with *C. helmsii*. At the single species level, *C. helmsii* and the dominant plant species *P. australis* showed a high capacity to coexist, although *C. helmsii* was most abundant where *P. australis* was absent. Based on these three experiments, evidence was not found to suggest that *C. helmsii* has a negative impact through direct displacement of native plants. However, because dominating *C. helmsii* growth may be more likely to occur in an area with ample bare ground into which this species can spread, and where tall competitive plant species (e.g. *P. australis*) are in low abundance, management activities designed to increase species diversity by creating patches of open vegetation structure, might instead just encourage a higher abundance and dominance of *C. helmsii*.

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Acknowledgements

First and foremost I would like to thank my supervisory team, Anita Diaz, Rudy Gozlan, and John Day. Thank you for your guidance, expertise, and patience. Special thanks to Ant for acknowledging that I am not a "typical academic", which made all the difference.

I am indebted to Bournemouth University, the National Trust, and the Royal Society for the Protection of Birds for funding this research project.

From Bournemouth University I wish to thank Iain Green for extensive help with laboratory work, Harry Manley for dPGS instruction, Karen Walmsley for logistics, and the Applied Sciences administration team, especially Louise Pearson.

I am grateful to the New Forest Park Authority for allowing me to survey ponds in the New Forest, and to Naomi Ewald for sharing the locations of *C. helmsii* invaded ponds. Thank you to Barry Yates for allowing site access at Castle Water and Pett Pools, and for collaborating on the salinity experiment paper. To Jacqui Miller and Ricky Whelan for site access at RSPB Fen Drayton, to Chris Hudson for site access at RSPB Ouse Fen, to Roger Beecroft and James Page for access at Kingfishers Bridge and for allowing many return visits. I would like to thank all of the members of the reserve team at Studland Nature Reserve (National Trust), particularly Armand Ian Delbarre, Angela Peters, and Aemelia Roe for their help in initiating the experiment at Little Sea and for their skippering skills.

Thank you to everybody who has helped me over the years with fieldwork: Clare Davies, Daniel Doherty, Ella Egberts, Greg Murray, James Page, Vicky Penson, Sui Chian Phang, Ann Thornton, and Annalea White. I am indebted to everybody who kindly collected data on my behalf for use in chapter three: Paula Baker, Charlotte Bell, Toby Branston, Vicky Buckel, Paul Charlton, Andy Coulson-Phillips, Phil Dean, Cath Dewhurst, Amy-Jayne Dutton, Simon Ford, Leanne Harris, Natalie Holt, Peter Hughes, Sarah Money, Dave Morgan, Edward Nicholson, Rhian Pierce, Martin Randall, Jon Sibthorp, Katherine Stearne, Andrew Tuddenham, Jenny Wallace, Michael Walter, Rob West, and Andrew Upton.

I would like to thank Greg Murray for his endless positivity and good humour. Thank you to Phil and Sue Dean for their unwavering support, not just during this PhD, but during the whole of my university education. Thank you to the PhD community for the 'witty' banter, the climbing trips, and the occasional half marathon; I wish you all the very best of luck in your future careers. Finally, thank you to Jemima, Lola, Maurice, and Angus for really not caring whether or not I finished my thesis.

Author's declaration

1) I confirm that the work presented in this thesis is my own work, with the following exception: Included in chapter three, are data of *C. helmsii* invaded ponds which were surveyed as part of an unconnected research project in which the author of this thesis was not involved (Sayer *et al*. 2012; Sayer *et al.* 2013). Data from this research project were provided for use here by Carl Sayer. In chapter three data were also voluntarily collected on behalf of the author, by reserves teams from the RSPB, the National Trust, the Wildlife Trusts (BBOWT), and Natural England.

2) Chapter four is published in collaboration with John Day, Rudy Gozlan, Iain Green, Barry Yates, and Anita Diaz.

Dean C., Day J., Gozlan R.E., Green I., Yates B. Diaz A. 2013. Estimating the minimum salinity level for the control of New Zealand Pygmyweed *Crassula helmsii* in brackish water habitats. *Conservation Evidence*. **10**. 89-92.

3) Chapter five is published in collaboration with John Day, Rudy Gozlan, and Anita Diaz.

Dean, C.E. Day, J. Gozlan, R.E. Diaz, A. 2015. Grazing vertebrates promote invasive swamp stonecrop (*Crassula helmsii*) abundance. *Invasive Plant Science and Management*. In Press. Available from: http://www.wssajournals.org/doi/10.1614/IPSM-D-14-00068.1.

1. Main introduction

1.1. Background

What is a non-native invasive plant?

A species is referred to as 'non-native' when it is found within a region which is outside the species' indigenous range (Hulme *et al.* 2008; Blackburn *et al.* 2011). Whilst species distributions can be naturally wide-ranging, the term non-native implies that the organism encountered the new region because human activity transported it beyond the geographical and physical barriers which had historically prevented its spread, for example across expansive oceans to new continents, and that it would not occur in the new region if humans had not initially introduced it (Theoharides & Dukes, 2007; Hulme *et al.* 2008; Blackburn *et al.* 2011). In some cases this transportation is intended because the species is a commercially valuable commodity, however other species are transported unintentionally either as a contaminant of the commodity or because they are attached to the transport vessel (Hulme *et al.* 2008). For higher plants, species are most frequently intentionally transported as commodities, and are grown in new regions as food crops or ornamental garden plants (Hulme, 2009; Keller *et al.* 2009).

There are several mechanisms by which non-native plants can be dispersed beyond designated growing areas such as gardens (Hulme *et al*. 2008). For higher plants, the pathways often involve the unintended 'escape' of plants or propagules, which can be via natural dispersal mechanisms like flowing water, or via human activities such as the transport of contaminated soil, or the disposal of plant material into adjacent wild habitats (Willby, 2007; Hulme *et al.* 2008; Keller *et al.* 2009). Of these escaped plants a small sample of propagules may disperse to areas with abiotic conditions suitable for growth, and of these a small sample may overcome competition from the resident plant community and attack from natural enemies to grow to full maturity (Richardson *et al.* 2000; Henderson *et al*. 2006; Theoharides & Dukes, 2007). A non-native plant species which can grow to maturity and produce viable dispersing propagules in the wild, unaided by human cultivation, is said to be 'naturalised' (Richardson *et al.* 2000; Henderson *et al*. 2006; Pyšek & Richardson, 2006). Of the non-native species capable of becoming naturalised in a new region, a small number are able to successfully disperse to and become naturalised in multiple locations and at long distances from the source of escape, leading to the establishment of the species as constituents of the plant community across an increasingly wide scale. Such species are termed 'invasive' (Richardson *et al.* 2000; Pyšek & Richardson, 2006), particularly if they characteristically attain high relative abundance in the areas where they become naturalised (Henderson *et al*. 2006). Thus there is a distinguishable

process which starts with the transport of plants to a new region, and results in a small number of these species becoming invasive (Richardson *et al.* 2000). It should be noted however that this process is not linear; the same species can be transported on multiple occasions, cultivated in several different areas, and escape into the field many times via different pathways, leading to multiple independently arising naturalised populations of the non-native species (Davis, 2009).

In this thesis the term 'non-native invasive plant' is defined as meaning a non-native plant species which has naturalised across a wide geographic scale in a non-indigenous region, and which readily colonises newly encountered suitable habitat, where it can display invasive growth. The term 'invasive growth' is defined in this thesis as meaning the accumulation of highly abundant and prolifically spreading biomass, within a colonised habitat.

Why do some non-native plant species become invasive?

The identity of species which successfully naturalise and become invasive varies between regions, and the same species introduced to several separate regions can have differing invasion success (Heger & Trepl, 2003; Davis, 2009). This variation in the outcomes from non-native species introductions has prompted research, to try and understand the principle factors determining which non-natives become invasive, where, and why (Richardson & Pyšek, 2006). Much of this research has been directed towards understanding how the abiotic environment and interactions with other organisms, and the specific traits of a non-native plant, combine to determine whether the plant will become a successful invader within a particular region.

In a heterogeneous landscape, the likelihood of a non-native plant becoming invasive will vary according to how favourable the abiotic conditions are for the development of prolific growth and spread (Meekins & McCarthy, 2001; Wang *et al.* 2006; Cheplick, 2010; David & Menges, 2011; Warren *et al.* 2011; Kostrakiewicz-Gierałt & Zając, 2014). For instance, Andrews *et al.* (2009) suggested that *Impatiens glandulifera* could become invasive within deciduous woodland in situations where a fallen tree creates transient conditions of higher light and nutrient availability. The authors explained that the ability of *I. glandulifera* seeds to respond quickly to increased light and nutrient levels, with rapid and extensive germination, meant that it could out-compete other woodland floor plants under such conditions. Whilst there are exceptions (Funk & Vitousek, 2007), increased resource availability may be a key environmental parameter influencing invasion success in a large number of cases. In a review of published non-native invasive-versus-native comparison studies, Daehler (2003) concluded that non-native invasive species often performed (in terms of fitness) better than the natives only under certain growth conditions or at certain life stages; in particular a number of non-native invasive species performed better with high nutrient levels. Such findings concur with the 'fluctuating resource availability theory' (Davis *et al.* 2000) which proposes that variation in invasion success is, in general, related to variation in the availability of resources. That the

availability of resources is heterogeneous over time and across space, and that the likelihood of invasion is greatest during those occasions and in those locations where resource availability is high. In a subsequent assessment of this theory Davis (2009) also points out that not all resources may have the same impact on invasive success. Thus attention needs to be given to determining which resource (e.g. particular nutrients, light, water, physical space) is more limiting to the further spread of a non-native species in a particular situation.

The likelihood of a non-native plant becoming invasive will also vary according to the outcomes of interactions with other organisms within the new region (Richardson & Pyšek, 2006). Biotic resistance describes the ability of a resident community to prevent or limit the invasive spread of a non-native (Davis, 2009), and is a topic that has received attention from researchers; in a meta-analysis Levine *et al*. (2004) reported that the spread of non-native plants may be limited, if not prevented, by competition. Other single-study papers however, have found competition to be a relatively weak factor limiting non-native plants compared to the abiotic environment (Detheir & Hacker, 2005; Thomsen *et al*. 2006; Gerhardt & Collinge, 2007), therefore there are still questions regarding the importance of resident plant competition as a factor mediating invasive success. Contrastingly, invasive success can be facilitated by interactions with the resident plant community. For example Smith *et al*. (2004) found that the seedling establishment of a non-native invasive forb (*Melilotus officinalis*) was positively associated with the abundance of a group of dominating grass species. The authors discussed how this result may reflect a facilitative interaction, in which the dominant grasses shielded the establishing seedlings from stressful levels of sunlight, and thus reduced mortality in the establishing non-native. At other trophic levels, it has been suggested that natural enemies such as herbivores and pathogens could exert biological resistance, if they caused biomass loss or increased mortality in a non-native plant (Levine *et al*. 2004). Herbivory experiments have shown that non-native invasive plants can suffer equal or greater damage than natives or non-invasive plants (Agrawal & Kotanen, 2003; Stricker & Stiling, 2012; Fan *et al*. 2013), although it has been disputed whether such impacts on individual plants, translate to a limitation on the invasive spread of the population (Maron & Vilà, 2001). Conversely, if the non-native plant experiences lower levels of attack (Keane & Crawley, 2002; Mitchell & Power, 2003), or is less affected by damage from natural enemies compared to the resident plant species (Kimball & Schiffman, 2003; HilleRisLambers *et al.* 2010), this could facilitate invasive spread. Overall there are few generalisations that can be made about how interactions with other organisms affect invasive success. It is therefore valuable to conduct research on particular non-native species, and particular interspecific interactions, in order to understand the contexts in which such interactions facilitate or suppress invasive success.

It has been recognised that the traits of a non-native species may or may not be advantageous, based on the abiotic and biotic conditions of the region (Daehler, 2003; Heger & Trepl, 2003;

Pyšek & Richardson, 2006; Davis, 2009). However, there has also been investigation of whether successful non-native invasive species possess specific traits which made them inherently more able to overcome the abiotic and biotic barriers lying between escape, naturalisation, and invasiveness (Kolar & Lodge, 2001; Heger & Trepl, 2003). Indeed from single-system studies, researchers have identified traits which likely aided in invasive success (Allred *et al*. 2010; Marco *et al*. 2010). For example, Bohl Stricker & Stiling (2013) concluded that the non-native invasive shrub *Eugenia uniflora* had become a successful invader in Florida (U.S.) because more numerous seedling emergence and a taller stem height had allowed it to out-compete both native and non-invasive non-native species for access to resources during establishment. Comparisons between closely related invasive and non-invasive species have also identified traits related to invasiveness within a particular taxon (Grotkopp *et al*. 2002; Burns, 2004; Keser *et al.* 2014; Skálová *et al*. 2013). At the wider scale, research has been directed towards determining whether a general suite of traits exists, which could be used to predict invasive success across a diverse range of non-native invasive taxa (Kolar & Lodge, 2001; Davis, 2009). For example, analyses of the literature have found that successful non-native invasive plant species tend to possess traits relating to better competitive performance, such as a high relative growth rate (van Kleunen *et al.* 2010; Dawson *et al*. 2011) or large size and height (Williamson & Fitter, 1996; van Kleunen *et al.* 2010), and also traits relating to efficacious dispersal (Williamson & Fitter, 1996; Kolar & Lodge, 2001; van Kleunen *et al.* 2010). However, there is nevertheless considerable variation in the suite of traits possessed by successful plant invaders (Grime, 2001; Heger & Trepl, 2003), and no particular traits have been discovered which are essential for invasive success (Heger & Trepl, 2003). It has also been suggested that invasive success may be enhanced if a non-native plant can exhibit plasticity in its traits, in response to varying conditions (Daehler, 2003). Indeed, Martina & von Ende (2012) hypothesise that the non-native invasive grass *Phalaris arundinacea* has been able to successfully invade habitats across the majority of the United States, because it displays phenotypic plasticity in its traits relating to growth and resource allocation, and so can remain competitive under changing conditions of light, nutrient, and moisture availability. Again however, it has not been possible to draw general conclusions about the level of such phenotypic plasticity exhibited by successful non-native invaders; non-native invasive plants do not consistently have higher phenotypic plasticity than native or non-invasive congeneric species (Palacio-López & Gianoli, 2011), and the possession of phenotypic plasticity may not necessarily be the cause of invasive success (Davidson *et al*. 2011).

What are the ecological impacts of non-native invasive plants?

A major concern regarding non-native invasive plants is that they may competitively suppress or exclude native plant species, ultimately causing declines in species diversity (Levine *et al.* 2003; Vilà *et al.* 2011). Indeed studies have shown that non-native invasive plants can suppress native competitors at germination (Greene & Blossey, 2012), during vegetative growth (Murrell *et al*. 2011), and at the reproductive stage (Gooden *et al*. 2014). Furthermore, many non-native invasive plants attain wide distributions within invaded regions (Stohlgren *et al.* 2011), which has lead to concerns that ubiquitous invaders may replace scarce or locally distributed native species, and thus contribute towards the biotic homogenisation of regions (Schwartz *et al.* 2006; Winter *et al*. 2010; Chen *et al.* 2013). The initial invasion of one species may also facilitate the subsequent colonisation of other non-native invasive species (Simberloff & von Holle, 1999). For example Santos *et al.* (2011) suggest that the invasion of the non-native aquatic plant *Myriophyllum spicatum* into Californian river delta systems created shallower areas of the river bed which then aided colonisation by the aquatic non-native invasive plant (*Egeria densa*), which has now come to dominate this system.

Invasion by a non-native plant, which causes reductions in native plant species, may in turn also cause reductions in species from other trophic levels if they are dependent on interactions with the native flora (Levine *et al*. 2003; Keeler *et al*. 2006; Gerber *et al*. 2008). Alternatively, plant invasion can have a direct impact on non-plant organisms if invasion leads to changes in the physical or abiotic characteristics of the habitat (Hessen *et al*. 2004; van der Wal *et al*. 2008; Stiers *et al*. 2011). For example Schultz & Dibble (2012) discuss how the invasion of a non-native plant to aquatic systems may impact upon fish and invertebrate communities through changes to the structure and complexity of submerged habitats, impeding foraging and restricting the movement of larger individuals. Non-native plant invasion can also affect ecosystem functioning, in cases where the invader possesses significantly different functional traits to the plants that it replaced (Levine *et al*. 2003; Simberloff, 2010). For instance if a non-native invasive plant differs significantly in the amount of available nutrients it contributes to the system, compared to the previously occupying natives, as a primary producer this can have a strong effect on the rate of nutrient cycling within an ecosystem (Yelenik *et al.* 2004; Ashton *et al.* 2005; Simberloff, 2011).

Whilst ecosystem impacts caused by the invasions of non-native plants have been detected in many cases, there is considerable variation in the magnitude of these impacts (Ortega & Pearson, 2005; Vilà *et al*. 2011; Pyšek *et al*. 2012b). For example, a high impact invasion was reported by Gerber *et al.* (2008) who found a negative association between the abundance of non-native invasive *Fallopia* species, and the diversity of native plant species, which the authors suggested was in turn driving a decline in the abundance and richness of phytophagous invertebrates. In contrast a low impact invasion was reported by Meffin *et al*. (2010), who found

no negative association between the abundance of a non-native invasive herb (*Hieracium lepidulum*) and native plant species diversity or evenness over a six year study period, instead finding evidence for coexistence between the non-native invasive and native species. An important area of research is therefore to investigate why some non-native invasive plants have greater impacts than others.

Equally, research is needed to understand why there might be variation in the level of impact caused by the same non-native invasive plant. It has been recognised that the ability of a non-native invasive species to competitively suppress native species is context dependent (Ortega & Pearson, 2005; Pyšek *et al*. 2012b). As such, interactions can be affected by the traits of the non-native invasive plant relative to the traits of the native plants. This was shown by Hedja *et al.* (2009), who assessed 13 different non-native invasive plant species for their impact on species diversity and evenness, and found that some non-native invasive species reduced diversity and evenness whilst other non-native invasive species had little or no impact. The authors attributed these results to the differential ability of the 13 species to overtop competitors, with the largest impacts seen when the non-native invasive species showed taller, denser growth which could shade out smaller native species. Competitive interactions can also be mediated by the characteristics of the habitat, for example a non-native invasive species may have a reduced competitive ability when abiotic conditions are less favourable for its growth (Price *et al*. 2011; Warren *et al.* 2011; Goldstein & Suding, 2014). Wang *et al.* (2006) described a gradient of suitability within a salt marsh habitat, and showed experimentally that a non-native invasive wetland grass (*Spartina alterniflora*) had increased dominance at high water levels and high salinity, whereas this non-native invasive species was dominated by a native species (*Phragmites australis*) in drier and less saline conditions. Such context dependence is also shown for impacts related to ecosystem functioning (Simberloff, 2011). For instance invasion by a nitrogen fixing species would likely have a smaller impact on nutrient cycling in habitats which are already naturally high in nitrogen (Levine *et al*. 2003).

Importantly, such examples demonstrate the overlap between research to understand where a non-native invasive plant has the greatest impacts, and research to try and understand where a non-native plant will be most invasive; both are mediated by the abiotic and biotic conditions of the region. Due to the lack of general patterns, research which focuses on a single non-native invasive species is still highly valuable, as it is necessary to understand something of the ecology of an individual species in order to understand why it has become invasive, and whether its interactions with the native species community are likely to result in negative ecosystem impacts.

Control of non-native invasive plants

In order to ameliorate the negative effects non-native invasive plants can have on ecosystems, it is often deemed necessary to try and eradicate them or to control their growth. Eradication is generally considered to be the most desirable outcome (IUCN, 2000), completely removing the chances that a non-native invasive plant could have future negative ecosystem impacts. There have been some successful eradication schemes, with greater success seeming to be had from attempts over small spatial scales (Rejmánek & Pitcairn, 2002; Genovesi, 2005; Simberloff, 2009a), but also many failures (Manchester & Bullock, 2000). Rejmánek & Pitcairn (2002) discuss that it is often unfeasible to completely eradicate a non-native invasive plant which has become widespread over large spatial scales (greater than 10,000 hectares), and Davis (2009) argues that small scale eradication may also be unsuccessful over the longer term, due to the chances of re-invasion from surviving non-native populations in the surrounding landscape. In such cases control schemes are often nevertheless implemented, with the aim of limiting the abundance and spread of non-native invasive plants, in order to reduce their potential to have negative ecosystem impacts (IUCN, 2000; Simberloff, 2009a).

Methods commonly used for control, focus on the removal of biomass by hand or with machines, or focus on the degradation of biomass in situ, for example by burning or spraying with chemical herbicides (Charudattan, 2001; Environment Agency, 2010). The idea behind such control methods, is that by removing the non-native invasive plant, native plants in the community will have time to recover and fill the space previously occupied by the invader. In accordance with this Jäger & Kowarik (2010) reported the successful regeneration of the native Galapágos plant community following the removal of the non-native invasive tree species *Cinchona pubescens*, however the authors noted that some non-native species also benefitted from the control actions. Importantly, a criticism of such clearance methods is that they represent a disturbance event which creates conditions of increased resources; such activities would be likely to favour those species which are best able to take advantage of the available resources, which may not necessarily be the plant species which existed in the community before invasion (Hobbs & Huenneke, 1992; Mason & French, 2007). This is illustrated by Hulme & Bremner (2006) who reported that the plant species which most benefitted from the removal of the non-native invasive plant *Impatiens glandulifera* were common ruderal species which were adapted to colonising areas of recent disturbance. There is a need for more research which assesses the ability of a non-native invasive plant to recolonise following clearance control, and compares this to the ability of native species to recolonise. This information could inform land managers of whether clearance control methods are likely to be an effective way of removing a particular invader and encouraging the regrowth of desired native plant species.

An alternative to removal and degradation is to limit the abundance of a non-native invasive plant, and thus its ability to spread, by increasing attack from natural enemies. Classical

biological control; introducing into the invaded region, specialist natural enemies from the non-native invasive species' indigenous region (Cronk & Fuller, 1995); has been highlighted as an option for limiting non-native invasive plant abundance (Gassman *et al*. 2006; Sheppard *et al.* 2006). Biological control benefits from being a cost-effective and long term option, which if implemented carefully does not damage co-occurring native plants (Culliney, 2005), and has shown control efficacy in a number of cases (Van Driesche *et al*. 2010). That said, limiting a non-native invasive plant using this method still does not guarantee that the desired native species will benefit, for instance Stephens *et al.* (2009) reported that the decline in the non-native invasive plant, *Centaurea diffusa*, following the release of a biological control agent lead to subsequent increases in the abundance of non-native invasive grass species. Biological control attempts also require considerable preliminary research, due to the necessity of minimising the risk that the control agent could also attack non-target native plants (Barrat *et al.* 2010). Apart from the use of specialist natural enemies (classical biocontrol), it has been discussed how biotic resistance to non-native invasive plants could be strengthened in habitats by increasing grazing pressure from generalist herbivores (Popay & Field, 1996), or by encouraging the growth of strong native plant competitors (DeWine & Cooper, 2010; Denton, 2013). It has been emphasised here, how the outcomes of interactions between non-native plants and resident plants and herbivores are highly context dependent; to effectively use biotic resistance to control a non-native invasive plant, research would need to be conducted to assess whether particular plant or herbivore species interact with the the non-native in a way to limit its abundance, rather than facilitate its abundance. It has also been suggested that a non-native invasive plant could be limited or eradicated if abiotic conditions were made less suitable for its growth (Davis, 2009). For instance, Price *et al*. (2011) discussed how land management to encourage more frequent and persistent flooding, could be used to control the abundance of the non-native invasive grass *Phyla canescens* within a study region, because the species is competitively subordinate to the native grass *Paspalum distichum*, under flooded conditions. Further research to understand the environmental tolerances of individual non-native invasive plants is therefore another valueable line of further inquiry, and could be used to aid in the design of successful control attempts.

Summary of research themes

Understanding why some non-native plants produce highly invasive growth is a fundamental question in invasive species ecology (Elton, 1958; Cronk & Fuller, 1995), and provides valuable insights regarding the ability of non-native invasive plant species to negatively impact invaded ecosystems (Levine *et al*. 2003). Making a contribution to knowledge in this area requires investigation of, how the characteristics of the habitat influence invasive growth (Cheplick, 2010; Jauni & Hyvönen, 2012), how resistance from the native species assemblage mediates

invasive growth (Maron & Vilà, 2001; Corbin & D'Antonio, 2004), whether invasive growth is associated with specific changes in the habitat conditions or species assemblage (Davis *et al.* 2000; MacDougall & Turkington, 2005; Baiser *et al.* 2008), and how invasive growth is influenced by the specific traits of the non-native invader (Hejda *et al*. 2009; Marco *et al*. 2010; Jauni & Hyvönen, 2012).

Understanding why a non-native plant produces invasive growth can be of utility when making predictions regarding where a non-native plant is likely to be most invasive and dominating (David & Menges, 2011; Warren *et al*. 2011). Determining where a non-native plant is most likely to become invasive and dominating, allows management resources to be allocated to situations where preventing invasion or limiting abundance is most needed in order to limit the negative impacts of invasions (D'Antonio *et al*. 2004).

The research presented in thesis focuses on the non-native invasive plant species *Crassula helmsii*. Using this species, questions are explored which relate to understanding why a non-native plant species can become invasive, what the ecological impacts of invasion are, and how such information can be used to design effective future control schemes.

1.2. Study species: *Crassula helmsii* (T. Kirk) Cockayne

Morphology and growth habit

Crassula helmsii is a perennial herb which is adapted to grow within water or in wet areas adjacent to water. This species characteristically produces dense mats of low-growing vegetation cover. It has round stems which grow erect or prostrate, and which are simple to twice-branched. The stems can vary in maximum length from 100 mm to 1.3 m, depending on the depth of water. The stalkless leaves grow in opposite pairs along the stem and are linear to lanceolate in shape, growing to approximately 20 mm long and 2 mm wide. The flowers are produced singly on stalks from the leaf axils and are up to 4 mm in diameter, with four petals arranged in a star shape which are white to pale pink in colour (Laundon, 1961; Dawson & Warman, 1987; Sainty & Jacobs, 2003).

The growth form of *C. helmsii* varies depending on the depth of water in which it occurs. This species is plastic in its growth form, allowing it to respond to changes in water levels (EPPO, 2007):

Marginal growth: When occurring on land around the margins of water *C. helmsii* has a fleshy, succulent appearance. It produces prostrate and creeping stems which spread out across the ground, but can also form dense stands of erect stems up to 100 mm tall (Laundon, 1961; Dawson & Warman, 1987; CAPM, 2004; EPPO, 2007) (Fig. 1).

Emergent growth: When growing in shallow water (less than 0.6 m), *C. helmsii* grows up out of the water on erect stems, which it can produce in very dense stands (Laundon, 1961; Dawson & Warman, 1987; EPPO, 2007).

Submerged growth: In deeper water *C. helmsii* grows from a basal rosette which is rooted to the bottom substrate. It produces longer stems, and has narrow non-fleshy leaves which are more sparsely located along the stem. *Crassula helmsii* does not produce flowers on submerged stems (Laundon, 1961; Dawson & Warman, 1987; EPPO, 2007) (Fig. 1).

In all three growth forms *C. helmsii* is an evergreen plant, and experiences little die-back or dormancy during the winter (Preston & Croft, 1997; CAPM, 2004). However, it may be more resilient to winter conditions when submerged (Dawson & Warman, 1987). *Crassula helmsii* uses Crassulacean Acid Metabolism (CAM); a photosynthetic pathway in which carbon dioxide is obtained during the night and stored as malic acid, for use in photosynthesis during the day. It is hypothesised that CAM may provide *C. helmsii* with a competitive advantage in conditions where carbon availability limits photosynthesis (Newman & Raven, 1995; Klavsen & Maberly, 2009).

Figure 1. The appearance of the leaves of *Crassula helmsii* when the plant is growing terrestrially along the margins of waterbodies (top), compared to the appearance of the leaves when *C. helmsii* is growing submerged within water (bottom).

Reproduction

In its invaded range *C. helmsii* spreads clonally via above-ground mechanisms. Stands of *C. helmsii* vegetation increase in size by creeping stems which grow out into adjacent space and establish by producing roots and shoots from the stem nodes (Laundon, 1961; Hill *et al.* 2004). Dispersal over longer distances occurs by the transport of vegetative fragments. These fragments can be created when established stems of *C. helmsii* are broken up in a disturbance event (Dawson & Warman, 1987; CAPM, 2004; Hill *et al.* 2004; EPPO, 2007), and this species can produce small apical stem fragments which detach from the main stems in autumn (Preston & Croft, 1997; EPPO, 2007). It has been shown that *C. helmsii* can grow and establish from fragments of stem which contain just a single node, but not from single leaves, and that regeneration from shorter stem fragments may show particularly vigorous regeneration (Dawson & Warman, 1987; Hussner, 2009).

In southern England in August and September, *C. helmsii* produces flowers on emergent and terrestrially growing stems, which have a sweet scent (Dawson & Warman, 1987) and attract flying insects (personal observation). It is generally considered that *C. helmsii* does not reproduce sexually in the invaded range, because the seeds that it produces are non-viable (Dawson & Warman, 1987). Denys *et al.* (2014) report that seeds from *C. helmsii* growing wild in Belgium showed some viability however; in laboratory controlled germination studies where 300 flowers (maximum potential of two to five seeds per flower) were planted in shallow sandy substrate, 21 plants developed.

Habitat

Crassula helmsii is native to southern Australia and New Zealand. In its native range it is associated with swampy conditions, growing within and around the margins of still and slow flowing waterbodies, inland and in coastal habitats (Laundon, 1961; Dawson, 1989; Sainty & Jacobs, 2003).

Similarly in its invaded range *C. helmsii* has been recorded in ponds, flooded sand and gravel pits, lakes, reservoirs, marl pits, dune slacks, canals, ditches, fens, marshes, peat bogs, coastal lagoons and grazing marsh (Preston & Croft, 1997; EPPO, 2007; Charlton *et al.* 2010; personal observations). In these habitats *C. helmsii* has been found growing on damp ground along the margins of waterbodies, and within water down to depths of approximately 3 m. This species can tolerate water chemistry from acid to alkaline conditions and from low to high nutrient levels. It mainly inhabits freshwater but does grow in brackish conditions (Preston & Croft, 1997; EPPO, 2007), and is also tolerant to high concentrations of copper (Küpper *et al*. 2009). *Crassula helmsii* has not colonised fast-flowing waterbodies such as rivers (EPPO, 2007), and is

generally absent from locations where the substrate is exposed to frequent wave-action (Dawson & Warman, 1987; Preston & Croft, 1997; personal observations).

Invasion history

In Britain *C. helmsii* is naturalised across much of England and Wales, and has been recorded in scattered locations in Scotland and Northern Ireland (Fig. 2). This non-native plant has also invaded a number of other countries in Western Europe (EPPO, 2007; Minchin, 2008; van Valkenburg & de Hoop, 2013).

The population of *C. helmsii* in Britain originated from Tasmania (EPPO, 2007), and was first introduced in the 1910s (Dawson & Warman, 1987; EPPO, 2007). An early account of *C. helmsii* in Britain by Laundon (1961) describes how a plant retailer based in Enfield, Middlesex had been selling this species since 1927 as an oxygenating aquatic for use in outdoor ponds. Laundon (1961) also described several outdoor ponds in south east England where *C. helmsii* had naturalised. Dawson & Warman (1987) comment that *C. helmsii* was available to buy from many other plant retailers across England during the 1970s and 80s, and in fact legislation which banned the sale of *C. helmsii* in England and Wales did not come into force until April 2014 (Plantlife, 2014). Thus the trade of this species as an ornamental pond plant has likely greatly aided its spread across Britain (Keller *et al*. 2009), and the release of vegetation from garden ponds into the countryside may have facilitated the escape of this species into wetland habitats (Willby, 2007). The spread of *C. helmsii* in the countryside may have been further aided by the accidental transferral of vegetative fragments between waterbodies; it is thought that this transferral has been largely due to human activities, for example using the same fishing, pond dipping, or management equipment in many different waterbodies (Dawson & Warman, 1987; EPPO, 2007; Burchnall, 2013). It has also been speculated that large grazing animals could transfer fragments over short distances on their hooves (Dawson & Warman, 1987; Ewald *et al*. 2010), and that fragments could be spread over longer distances by waterfowl. Denys *et al* (2014) showed experimentally that it is possible for a vegetative fragment of *C. helmsii* to survive and grow after passing through the gut of a waterfowl, though such an event may happen only rarely.

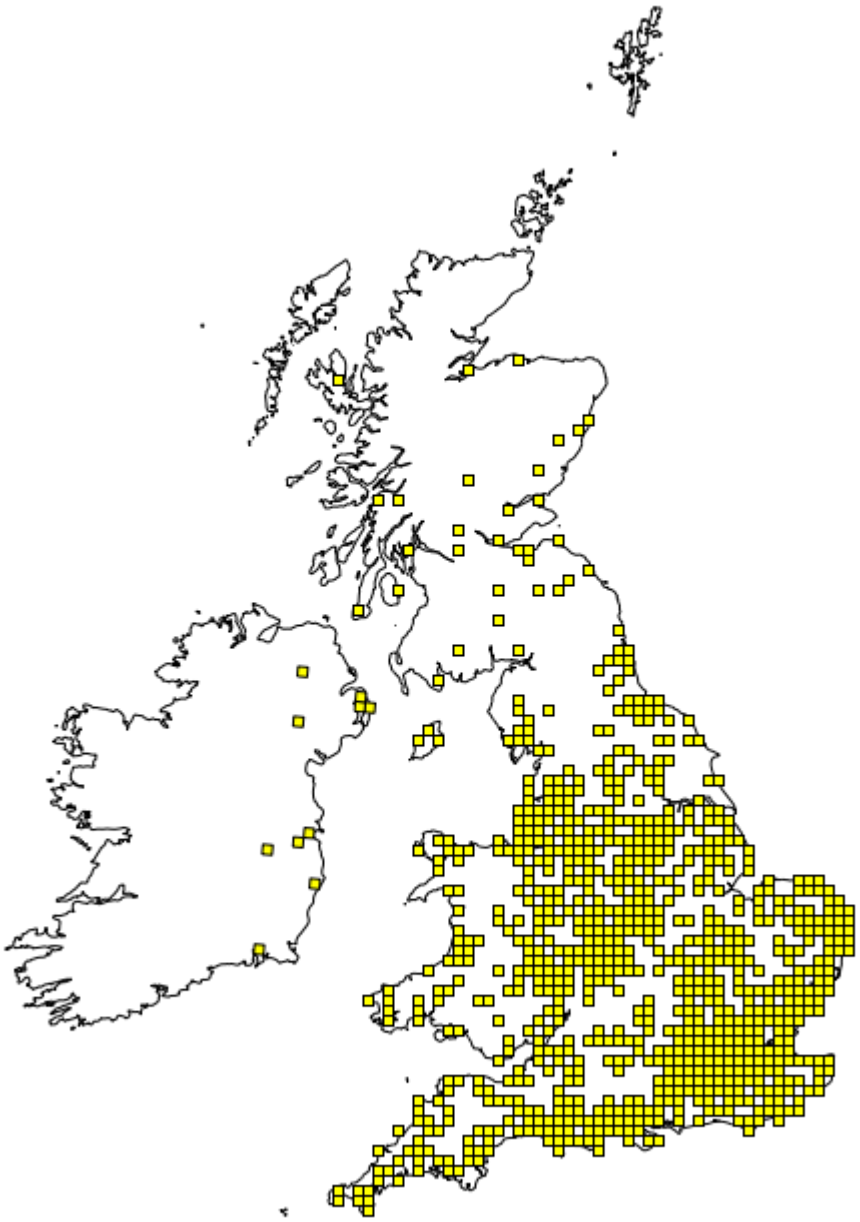


Figure 2. The distribution of *Crassula helmsii* in Britain, shown at a resolution of 10 km squares, and containing data from records up to 2014. Data courtesy of the NBN Gateway with thanks to all the data contributors: https://data.nbn.org.uk/Taxa/NBNSYS0000004639/Grid_Map (accessed 11th March 2014). The NBN and its data contributors bear no responsibility for the further analysis or interpretation of this material, data and/or information.

Impacts of invasion

Crassula helmsii can form extensive mats of vegetation across the marginal and emergent zones of waterbodies; a trait which is characteristic of the species both in its native and invaded ranges (Dawson, 1989; Sainty & Jacobs, 2003). In some locations these mats grow to become extremely dense, with few or no other species occurring amongst the invasive vegetation. This can change the appearance of the habitat, especially when *C. helmsii* grows along the margins of waterbodies (Fig. 3). It is these dense monospecific mats which are the main basis for concern regarding this species in the invaded range (Dawson & Warman, 1987).

There are concerns that such vigorous growth may be indicative of a competitively dominant species, and that the dense mats of *C. helmsii* can smother small plants and suppress their growth (Dawson & Warman, 1987). Conservationists suggest that *C. helmsii* invasion could reduce the abundance of native vegetation, and could potentially exclude specific wetland species (Bridge, 2005; Gomes, 2005; Wilton-Jones, 2005), thus reducing diversity. For example, highlighted as vulnerable are *Baldellia ranunculoides* (Yates, B. personal communication) and *Teucrium scordium* (Page, J. personal communication), which are classed as 'near threatened' and 'endangered' respectively, according to the IUCN Red List definition (Cheffings & Farrell, 2005). By extension there are concerns that the development of dense *C. helmsii* mats could negatively affect other organisms in the ecosystem; by displacing plant species which other organisms rely on, by reducing the availability of bare ground and open water, or by locally depleting dissolved oxygen levels in waterbodies (Watson, 1999; CAPM, 2004; Langdon *et al.* 2004; Foster, 2010).

Anecdotal accounts of *C. helmsii* suppressing native flora exist in the literature (Dawson & Warman, 1987; Watson, 1999), however quantitative studies of the impact of *C. helmsii* on native flora are less common. In a laboratory experiment Langdon *et al*. (2004) measured the germination success of 11 native aquatic plant species, with and without a 1 mm thick cover of *C. helmsii* vegetation. The authors found that the germination success of six species was significantly reduced under the *C. helmsii* cover, and discuss that this may be indicative of the effect of *C. helmsii* cover on the germination success of native species in the field. However in the same paper, Langdon *et al*. (2004) found no significant decline in plant species richness in *C. helmsii* invaded ponds over a four to seven year period, although results were not provided for changes in species abundance over that time, and another germination experiment did not find a significant difference in the number of species in the seedbank between *C. helmsii* invaded and non-invaded sites.

Figure 3. The bright green patches in the photograph are dense mats of *Crassula helmsii*, which grows extensively along the exposed muddy banks at Castle Water, in Rye Harbour nature reserve, East Sussex (© Barry Yates).

Control

Crassula helmsii is naturalised and widespread to the extent that its total eradication in Britain is probably unfeasible (Rejmánek & Pitcairn, 2002) although there is evidence that *C. helmsii* can be eradicated from individual sites (Charlton *et al*. 2010). Because *C. helmsii* is classed as a non-native invasive species, land managers are encouraged to take action to control its abundance and spread (Environment Agency, 2010). Methods which are used for control include; dredging the marginal and emergent zone and removing subsequent dead vegetation (Environment Agency, 2010), covering the plant with black plastic for three to six months to prevent access to light (CAPM, 2004; Wilton-Jones, 2005; Environment Agency, 2010; van Valkenburg & de Hoop, 2013), spraying the vegetation with the herbicide glyphosate (Dawson, 1996; Bridge, 2005; Gomes, 2005; Environment Agency, 2010), and spraying the vegetation with 'hot foam' to kill *C. helmsii* through heat stress (Bridge, 2005; Pearson, 2012). These control attempts often only produce reductions in *C. helmsii* abundance however, leaving living *C. helmsii* vegetation which can re-colonise the habitat (Bridge, 2005; Gomes, 2005; Wilton-Jones, 2005; Denton, 2013; van Valkenburg & de Hoop, 2013). Indeed it may be valuable to consider whether the clonal traits of *C. helmsii*; creeping horizontal growth and the ability to regenerate from small stem fragments (Dawson & Warman, 1987; Hussner, 2009); make this species particularly well adapted for recovery after clearance control.

Greater success had been achieved with salt water inundation; *C. helmsii* was eradicated from 120 ha of coastal grazing marsh by draining the brackish water, allowing seawater to flood the habitat, and then keeping it inundated for 12 months (Charlton *et al*. 2010). This method was feasible because the site had near access to sea water, and such actions could prove logistically difficult to implement at sites further from the coast.

The above described options for controlling *C. helmsii* are all non-specific, meaning that any native plants and animals living in-amongst the invasive vegetation would also be subject to the effects of the control treatment. In this way non-specific control methods can be problematic, if negative impacts on non-target species hamper the recovery of the pre-invasion native species assemblage (Matarczyk *et al*. 2002; Kettenring & Reinhardt Adams, 2011). Classical biological control has been suggested as an alternative method for reducing the abundance of *C. helmsii* in the invaded range (CABI, 2013), and the introduction of a specialist natural enemy would ideally minimise the impacts of control on the native plant community (Cronk & Fuller, 1995). Research and development is on-going in this area (CABI, 2013), and so it is not yet known whether the release of biocontrol agent could reduce the abundance of *C. helmsii*. It has been noted that large vertebrates such as ponies (Ewald *et al*. 2010; personal observations), sheep and wildfowl (Cadbury, J. personal communication) may graze patches of *C. helmsii*, and therefore it may be useful to investigate whether these generalist herbivores could be an effective method of limiting *C. helmsii* abundance.

Why study Crassula helmsii?

Concerns regarding *C. helmsii* in the invaded range stem from its ability to form dense monospecific mats. However, such mats are not always observed and *C. helmsii* can be found in the field growing in a mixed stand alongside native species (Fig. 4). Further, it is not clear whether the development of dense *C. helmsii* mats is associated with synchronous changes in the resident plant community, such as declines in species diversity. More research on this species is required in order to better understand when it develops dense monospecific mats and why, and furthermore, whether the spread of dense *C. helmsii* cover can result in the displacement of native plants. Attempts to control *C. helmsii* have rarely been successful long-term; a greater knowledge of the ecology of this species may help to identify ways that the abiotic or biotic environment could be manipulated in order to maintain *C. helmsii* at lower abundances. Furthermore, if the factors which contribute towards the formation of *C. helmsii* mats are understood, specific habitats or nature reserves can be assessed for their vulnerability to *C. helmsii* domination, and control efforts can be targeted towards those sites or parts of sites, where control is most critical for suppressing this non-native invasive plant.

Figure 4. *Crassula helmsii* occurring as a dense and extensive monospecific mat of vegetation (top), and *C. helmsii* growing in a mixed stand alongside native species (bottom).

1.3. Research aim and objectives

The main aim of this thesis is to better understand the mechanisms which allow *C. helmsii* to develop dense, dominating vegetative mats. Towards this aim, aspects of the ecology of *C. helmsii* were investigated which were hypothesised to affect its growth rate and abundance; namely its response to abiotic variation, and its interactions with other plant species and with generalist herbivores. As such, this research offers an opportunity to assess how *C. helmsii* may have an impact on the ecosystems that it invades via its impact on native flora, and provides information which may be of utility when designing control schemes to limit the prolific growth of *C. helmsii*.

Within the main aim, five objectives were addressed which are presented in the form of research questions:

1. How well does abiotic variation predict variation in *C. helmsii* abundance in the field?

Field data were analysed to assess whether *C. helmsii* abundance varied in relation to pH, nutrient levels, water depth, and shade. This objective therefore, contributed to the overall aim by providing information on whether high *C. helmsii* abundance was associated with particular abiotic conditions.

2. What is the estimated minimum salinity level for the control of *C. helmsii* in brackish water habitats?

A mesocosm experiment was conducted to investigate what level of salinity was required to kill *C. helmsii*. Within the experiment, the growth rate of *C. helmsii* was assessed when exposed to levels of salinity from 0 to 8 parts per thousand. Thus this objective contributed to the overall aim because information was obtained on how *C. helmsii* growth, and thus the development of vegetative mats, could be limited by variation in the abiotic environment (salinity).

3. What is the effect of vertebrate grazing disturbance on the abundance of *C. helmsii* within a wetland plant community?

Grazing exclosures were set up within a heavily *C. helmsii* invaded nature reserve, in order to ascertain whether the presence of large vertebrate herbivores affected the abundance of *C. helmsii*. As such, this objective contributed to the overall aim by assessing whether interactions with generalist herbivores could mediate the abundance of *C. helmsii*.

4. Is available bare ground an important pre-requisite to high *C. helmsii* abundance?

Field and common garden experiments were conducted which assessed how well *C. helmsii* could compete for available bare ground, compared to native plant species occupying a similar niche. This objective contributed to the overall aim by considering how *C. helmsii* growth rate and abundance could be mediated, by the presence or absence of adjacently spreading native plants. This objective also provided information on whether *C. helmsii* could have an impact on invaded ecosystems by out-competing individual native plant species.

5. Can the invasion of *C. helmsii* negatively affect the plant community in a *Phragmites australis* dominated fen habitat?

Field survey data were collected of *C. helmsii* abundance, *P. australis* abundance, and the abundance and diversity of plant species in the co-occurring community. These data were analysed to ascertain whether *C. helmsii* was negatively correlated with other plant community variables; a pattern indicating that an increase in *C. helmsii* abundance was associated with the loss of species from the resident plant community. This objective contributed to the overall aim by looking for evidence that *C. helmsii* invasion could increase in abundance by displacing native species. This objective also offered an assessment of the impact of *C. helmsii* invasion on native plant diversity.

2. General methods and materials

2.1. Field surveys

This section describes the methodology used in a field survey of *C. helmsii* invaded habitat, and gives brief descriptions of each of the sites visited for this survey. The data gathered from this field survey are presented in chapters three and seven, and one site described here was also referred to in chapter five.

2.1.1. Sampling technique

Survey data were gathered from 94 sampling locations. These sampling locations occurred within 28 separate water bodies that were spread across five geographically separate survey sites (nature reserves), all located in southern and eastern England (Fig 6.). To reduce the magnitude to which dispersal could influence the presence or absence of *C. helmsii*, all survey sites were selected on the basis that *C. helmsii* was known to occur there. These sites were: Castle Water, Fen Drayton, Kingfishers Bridge, The New Forest (ponds), and Ouse Fen (see section 2.1.2. below for site descriptions).

The sites varied considerably in their overall size, and in the size and number of waterbodies which they encompassed, and so a stratified design was used which ensured that sampling effort remained constant across sites. This stratified design determined one sampling location per hectare, which consisted of a $1m^2$ quadrat, with a minimum distance of 25m between individual sampling locations. In order to ensure that the same visual method of vegetation cover estimation could be used in all cases, surveying was also restricted to the margins and shallows (0 - 50cm depth water) of waterbodies. This meant that the number of samples at each site was dependent on the total area of marginal habitat per reserve (Table 2), and that large waterbodies were sampled at many points along the margins whereas small ponds had just one sample point.

To minimise bias, the location of each $1m^2$ quadrat was selected prior to surveying using the following approach. First, a satellite photograph was obtained of each waterbody from Google Earth (© Google, 2014) or from the online resource UK Grid Reference Finder (http://gridreferencefinder.com/). Then a to-scale $100m^2$ grid was placed over these maps and each grid square which overlaid a section of marginal habitat was marked. For each of these grid squares a vector was generated using the random number formula in Excel 2010 (© Microsoft, 2010) to create X Y coordinates, which corresponded to the horizontal and vertical axis of the grid square when measured in millimetres. The patch of marginal habitat on the

photograph in closest proximity to the vector was selected as the sample point. Ten figure OS grid references were obtained for each of the selected locations using UK Grid Reference Finder. This allowed sample points to be navigated to in the field using a Garmin model GPS device (± 5m accuracy).

2.1.2. Survey sites

Castle Water

Castle Water is a wetland site located within Rye Harbour Nature Reserve, East Sussex (OS grid ref: TQ 926 187), which is owned by the Sussex Wildlife Trust. Castle Water is a freshwater system, with expanses of open water and reedbeds. Through the reedbeds also run water channels, which are bordered by exposed (not submerged) silty banks and margins of silt and gravel. *Crassula helmsii* was first found at the site in 2007 (Yates, B. personal communication). Sample locations for the described field survey (chapters three and seven) were selected from within the reedbed and water channel habitat, which covered an area of c. 12 ha. In 2012 when surveying was conducted, *C. helmsii* was growing extensively as a dense, almost monospecific marginal mat, across the exposed silty banks. *Crassula helmsii* was also growing along the silt and gravel margins, immediately adjacently to the reedbeds, and in amongst the reeds.

Fen Drayton

RSPB Fen Drayton Lakes (hereafter 'Fen Drayton') is a nature reserve in Cambridgeshire (OS grid ref: TL 352 680), which is owned by the Royal Society for the Protection of Birds (hereafter 'RPSB'). The nature reserve contains a series of lakes which have a varied marginal habitat, consisting of dense reedbeds, over hanging stands of willow, and more open patches with a shorter vegetation structure. *Crassula helmsii* is known to have been present at the site since at least 2008 (Shanklin, J. personal communication). Field survey sample locations were selected from an area of c. 62 ha, which encompassed two of the lakes. In 2012 *C. helmsii* was only found within the more open patches of marginal habitat, where it occurred as a mixed stand alongside other plant species.

Kingfishers Bridge

Kingfishers Bridge is a privately owned nature reserve in Cambridgeshire (OS grid ref: TL 540 732). This nature reserve contains a lake with a variable marginal habitat of reedbeds and open patches of shorter vegetation, and a water meadow with drainage ditches. *Crassula helmsii* was first detected in 2007, along the drawdown zone of the lake (Cadbury, J. personal

communication). Field survey sampling locations were selected along the margins of the lake, which covered an area of c. 9 ha. In 2012 *C. helmsii* occurred within the open vegetation along the lake margins at high abundance, and at lower abundance along the lake margins vegetated with reedbeds.

The New Forest (ponds)

The New Forest is a National Park in Hampshire, which is managed by the New Forest National Park Authority. The park encompasses broadleaf and coniferous woodland, dry heathland, grassland, wet heathland, and bog. Within these broad habitats are numerous ponds, some of which have been invaded by *C. helmsii*. This species was first recorded in The New Forest in the mid-1970s (Rand & Chatters, 2010). Fifteen separate *C. helmsii* invaded ponds were visited for the field survey (Table 1; Fig. 5), the locations of which had been provided by Dr Naomi Ewald (Freshwater Habitats Trust). The ponds included in the survey occurred within the grassland and wet heathland, and many were in close proximity to development such as roads, car parks, and houses. The ponds ranged from a seasonally flooded area of c. 60 m^2 to a large permanent pond of c. 0.5 ha, and were vegetated with low-growing emergent and marginal plant species. In 2012 there was considerable variation in the abundance of *C. helmsii* within these 15 ponds.

Table 1. The Ordinance Survey grid references of the 15 *Crassula helmsii* invaded ponds which were surveyed within The New Forest, Hampshire.

Pond	Grid reference
1	SU 23457 11911
2	SU 19920 02141
3	SU 17796 12882
4	SU 40546 04943
5	SU 37393 01755
6	SU 36964 01677
7	SU 36724 01706
8	SU 36839 01343
9	SU 30616 03497
10	SU 21824 03589
11	SZ 23532 98715
12	SZ 23903 98608
13	SZ 36808 97544
14	SZ 33413 98547
15	SZ 33735 98939

Figure 5. A satellite image of the New Forest, Hampshire, showing the spatial distribution of the 15 *Crassula helmsii* invaded ponds that were surveyed here. Image © 2015 Getmapping plc, © 2015 Infoterra Ltd & Bluesky, image © 2015 The Geoinformation Group.

Ouse Fen

RSPB Ouse Fen (hereafter 'Ouse Fen') is a RSPB owned nature reserve in Cambridgeshire (OS grid ref: TL 348 729). It consists of disused sand and gravel quarry pits, which are being converted into a series of five lakes, with open-water and reedbed habitat, surrounded by grassland. *Crassula helmsii* was first found at the site in 2007 (Hudson, C. personal communication). Field survey sample locations were selected from one lake of c. 17 ha, which was the first to be converted in 2004 (Hudson, C. personal communication). This lake is bordered by extensive reedbeds, with a few more open patches of shorter vegetation. In 2012 when surveying was conducted, *C. helmsii* was found growing abundantly through the reedbeds, as well as in more open patches.

Table 2. The number of individual sample locations ($1m^2$ quadrats) visited at each survey site, and the number of non connected water bodies which this sampling effort encompassed.

Survey site	No. of locations	No. of waterbodies
Castle Water	14	3
Fen Drayton	22	2
Kingfishers Bridge	9	1
The New Forest	29	15
Ouse Fen	20	1

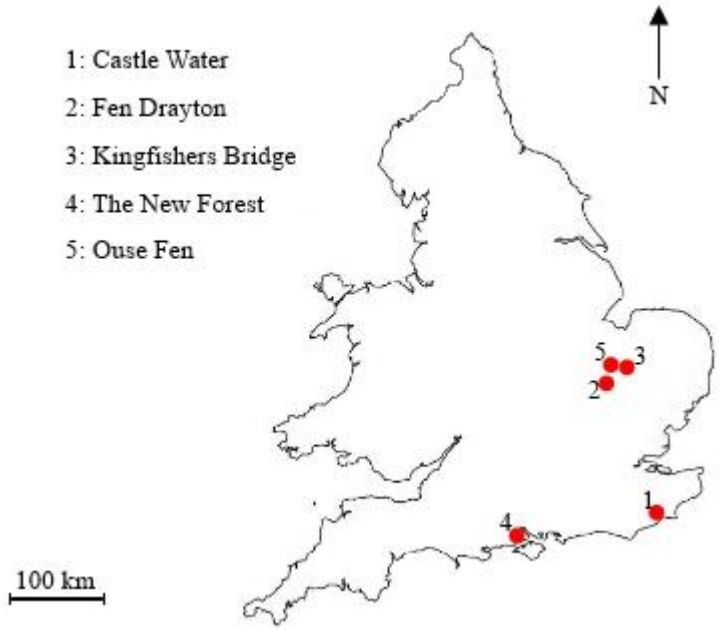


Figure 6. The geographical distribution of the five *Crassula helmsii* invaded sites (nature reserves), where surveying was carried out. These sites were: Castle Water, East Sussex; Fen Drayton, Cambridgeshire; Kingfishers Bridge, Cambridgeshire; The New Forest (ponds), Hampshire; and Ouse Fen, Cambridgeshire.

2.2. Data analysis

In this thesis diagnostic testing and statistical analysis was conducted using IBM© SPSS© Statistics version 20, unless otherwise stated.

2.2.1. Arcsine transformation

The arcsine transformation is a standard method that is employed to transform percentage or proportion data that is measured on a fixed scale, into data that do not deviate significantly from a normal distribution (Dytham, 2011; Krebs, 2014). In chapters three, five and seven, data on plant abundance were collected by estimating the abundance of each plant species on a fixed scale between 0 and 100%. Thus in order to normalise these data for parametric testing, an arcsine transformation was applied, utilising the formula:

=DEGREES(ASIN(SQRT('abundance'/100)))

In which 'degrees' converts radians to degrees, 'asin' is the inverse sine, 'sqrt' is the square root, and 'abundance' is the percentage cover of a particular plant species (Dytham, 2011). These transformations were conducted in Excel 2010 (© Microsoft, 2010).

2.2.2. Shannon-Weiner diversity and evenness

In chapters five and seven variables for the species diversity of the co-occurring plant community were produced. This was conducted in Excel 2010 (© Microsoft, 2010), using the number of species recorded in each sample location and their values for percentage cover to calculate the Shannon-Weiner diversity index (H), using the formula:

$$H = -\sum (P_i \log [P_i])$$

Where P_i is the proportion of an individual species relative to total vegetation cover, and log is the natural log. A variable for species evenness could then be produced by calculating the Shannon-Wiener Index of species evenness (J) using the formula:

$$J = H / \log (S)$$

Where S is the total number of species within a sample location, and log is the natural log (Begon *et al.* 2002).

3. How well does abiotic variation predict variation in *C. helmsii* abundance in the field?

3.1. Introduction

The habitat preferences of a species may be determined by using niche theory to interpret its response to environmental gradients (Hirzel & Le Lay, 2008). Niche theory predicts that the margins of a species' range represent the limits of its tolerance to certain environmental parameters, whilst the theoretical centre is characterised by optimum environmental conditions for survival and growth. As such, high abundance and vigour may be interpreted as a characteristic of plants growing within optimal conditions, whilst reduced abundance and vigour represent sub-optimal conditions (Leibold, 1995). This distinction between optimal and sub-optimal conditions is important as the outcome and strength of biotic interactions may vary according to the favourability of the conditions relative to different species' preferences (Silvertown, 2004).

Research into habitat preferences has a useful application in the study of non-native invasive plants, as it generates information about where species colonise and what limits their spread (Willis & Hulme, 2002; Jarnevich & Reynolds, 2011). For example Truscott *et al.* (2008) were able to define habitat patches in which the non-native invasive aquatic plant species *Mimulus guttatus* occurred, based on the abiotic parameters of available sediment and light, and stream water depth. Investigations of preference to environmental parameters therefore help to define the overall distribution of a non-native invasive plant (Thuiller *et al.* 2005; Albright *et al.* 2010), and this information can be used to make predictions of its potential to spread further across landscapes (Jarnevich & Reynolds, 2011; Gassó *et al.* 2009). A stronger synthesis between niche theory and investigations of non-native invasive plant spread may be achieved by inclusion of data regarding the optimal and sub-optimal environmental conditions within the habitable range of a species (Warren *et al.* 2011). Information can be obtained not only on where a non-native invasive plant species can grow but also where it grows best, and in this way key environmental gradients can be identified which affect the abundance as well as the presence or absence of the species (Meekins & McCarthy, 2001; Kilroy *et al.* 2008; Cheplick, 2010; David & Menges, 2011; Kostrakiewicz-Gierałt & Zając, 2014). Knowledge of the habitat optima of a non-native invasive plant species could therefore also be used to identify specific areas in which it may have a greater impact on the ecosystem (Huston, 2004; Wang *et al.* 2006; Davis, 2009).

Published information on the habitat range of *C. helmsii* documents a wide abiotic tolerance; *C. helmsii* can occur in acidic and alkaline sites, (Preston & Croft, 1997; EPPO, 2007; Minchin, 2008), in high and low nutrient conditions, (Preston & Croft, 1997), in sunlight and in shade (Hill *et al*. 2004), and can grow terrestrially and in water several metres deep (Preston & Croft, 1997; EPPO, 2007; Minchin, 2008). It is less clear from these sources whether there are known optima within this range, which are particularly favourable for *C. helmsii* growth. However, sources suggest that *C. helmsii* is more typical of higher nutrient conditions (Hill *et al*. 2004; Minchin, 2008), and a laboratory growth experiment found that the relative growth rate of *C. helmsii* increased with increasing soil nutrient levels (Hussner, 2009).

More research is required to understand how strongly *C. helmsii* abundance in the field is related to pH, nutrient levels, water depth, and shade. At present it is not clear whether optimal conditions of these abiotic variables are important in facilitating the growth of dense, dominant mats of *C. helmsii*, or whether *C. helmsii* can grow equally vigorously under most conditions. As such it is not known to what extent different components of the abiotic habitat are applicable when trying to predict how vulnerable a site is to *C. helmsii* domination. This chapter reports on a detailed field survey of the pH, macronutrient levels, water depth, and shading levels across five *C. helmsii* invaded sites in southern England. Analysis was conducted to test how much variation in *C. helmsii* abundance, was related to variation in these abiotic conditions.

3.2. Methods and materials

3.2.1. Field survey

A field survey was conducted between June 13th and September 6th 2012, in which 94 1m^2 sampling locations were visited at five geographically separate sites; Castle Water (E. Sussex), Fen Drayton (Cambridge), Kingfishers Bridge (Cambridge), The New Forest ponds (Hampshire), and Ouse Fen (Cambridge). See Chapter two, section 2.1 for site descriptions and an explanation of how the sampling locations were selected. The rationale for choosing to collect data from five geographically separate sites was that it allowed a greater range of abiotic variation to be encompassed within the field survey, than would have been possible if just one site had been surveyed more intensively. Within each sampling location, the abundance of *C. helmsii* was recorded as an estimate of percentage cover, and the following measurements were taken:

Water pH. This was measured using a Hanna pHep® hand held probe (accuracy at 20°C ± 0.1 pH) calibrated using pH 7.01 and pH 4.01 buffer solutions. Readings were taken by immersing the probe within water and waiting for the digital reading to stabilise. Three readings were taken at random positions within the quadrat area, and from this the mean pH value was calculated. Data on pH were obtained from 93 quadrats out of a total of 94 quadrats, as there was no water present in one quadrat at Ouse Fen.

Water samples. One water sample per quadrat was collected and stored in clean 60ml plastic sample pots for subsequent laboratory analysis of available nitrogen and total phosphorus concentration. Water was taken from just below the surface to avoid sediment disturbance during collection. In order to minimise post-collection changes due to chemical or biological reactions, all samples were kept in coolbags in the field and transferred as soon as possible (within 2 - 9 hours) to freezer storage (Radojevid & Bashkin, 2006). Water samples were obtained from 93 quadrats out of a total of 94 quadrats, as there was no water present in one quadrat at Ouse Fen.

Water depth. The depth was measured in centimetres, in four random positions within the quadrat. From these measurements a mean depth value was calculated. As described in Chapter two, section 2.1, surveying was restricted to water of 50 cm or shallower. However, this range represented the gradient from terrestrial to semi-aquatic habitat, and so was likely to encompass variation in abiotic conditions related to inundation.

Shading. A shade index was created by estimating the percentage of the quadrat that lay in the shadow of tall structures (greater tham 1 m), including vegetation, during mid-daylight hours. A limitation of this method is that it did not differentiate between intensity of shade created by

different structures, however it was deemed more appropriate than using a light meter to directly measure light levels. This is because surveying took place over a number of months, in different weather conditions and during different times of the day, and any light meter readings would have been influenced by these confounding variables.

3.2.2. Laboratory analysis

Water samples were tested for the concentrations of available nitrogen (nitrate, ammonia + ammonium) and total phosphorus. These macronutrients were chosen as they are considered to be most limiting to productivity in wetland ecosystems (Brönmark & Hansson, 1998; Dodds, 2002).

Total phosphorus in water. 10ml from each collected water sample was filtered through a 0.45μm syringe filter and transferred to a 15ml polypropene centrifuge tube. Total P concentration (mg / L) was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (NEMI$_a$, n.d.) using a Vista ICP-AES spectrometer. Readings were taken at a wavelength of 213.618 nm.

Nitrate (NO_3^-). Nitrate levels were determined using a uv / visible spectrometry method (NEMI$_b$, n.d.). 80μl of a water sample was mixed with 4ml of Griess reagent (VCl_3) within a plastic cuvette, and left for 12 hours for the completion of the chemical reaction. These solutions were then analysed for nitrate concentration (mg / L) using a Varian Cary uv-visible spectrometer.

Ammonia + ammonium (NH_3^- + NH_4^+). Levels were determined using an Ion Selective Electrode (ISE) method (NEMI$_c$, n.d.). 10ml from each water sample was mixed with 0.63ml of 2M NaOH, raising the pH in order to convert ammonium within the sample to ammonia. Ammonia concentration (mg / L) was then detected using a Thermo Scientific Orion 4 Star ISE portable probe.

3.2.3 Regional data

Before field data collection, it could not be exactly ascertained how much variation in pH and macronutrients was going to be encompassed by the survey. Therefore, to aid in interpretation of the results following data collection, pH and macronutrient data were obtained from a further 37 *C. helmsii* invaded sites in the UK (appendix I). This 'regional dataset' encompassed sites from a larger geographic distribution (Fig. 7), and provided pH and macronutrient measurements from a greater number of sites. The sample of pH and macronutrient levels in the field survey data could thus be compared to the sample in the regional dataset, as a way of

assessing whether the abiotic variation included in the survey, was similar to the variation recorded over a wider distribution.

The sites from which data were obtained were *C. helmsii* invaded areas primarily within nature reserves. Data were voluntarily provided by reserves teams from the RSPB, the National Trust, the Wildlife Trusts (BBOWT), and Natural England. Data were also provided from a detailed study of ponds at Manor Farm in Norfolk, UK which was unconnected with this research project (Sayer *et al*. 2012; Sayer *et al.* 2013).

Because data were not collected by the researcher, it was not feasible to repeat the stratified sampling method, as used for the main field survey. Instead abiotic data were collected 'per invaded site'. In this case a site was defined as an isolated waterbody such as a pond or lake, or an interconnected habitat such as a reedbed system, within which *C. helmsii* was growing.

In summer 2011, reserve team contacts were asked to test the pH of the water within each of the sites using Fisherbrand colour-fixed pH indicator sticks, which were provided by the researcher. This was considered to be the most appropriate method for recording pH as there was no technical equipment requiring calibration, and the indicator sticks were easy to interpret. Reserve team contacts were also asked to take one water sample per site, from the water within which the *C. helmsii* was growing, whilst taking care not to stir up sediment in the area where the sample was to be taken from. Contacts were sent 60ml plastic pots and blunt syringes with 0.22μm syringe filters for the purpose of taking water samples, and it was asked for water samples to be drawn into the syringe through the filter in order to remove large organic particles and large micro-organisms (Ward, 1997). This step was deemed as necessary in order to reduce nutrient degradation of the sample due to chemical or biological reactions (Radojevid & Bashkin, 2006). The syringe filters were then removed and the filtered water was transferred to the 60ml plastic pot, and posted back to Bournemouth University for laboratory analysis of nitrate, ammonia + ammonium and total phosphorus content, using the method described above.

Reserve contacts were also asked to provide categorical information on the abundance of *C. helmsii* within the site: 1) Forming dense carpets which exclude all other species, 2) highly abundant but other plants species still occur, 3) Frequent but not dominating the vegetation, 4) sparse or infrequent patches amongst other vegetation. Data from Manor Farm, Norfolk (Sayer *et al*. 2012; Sayer *et al*. 2013) were collected as part of research into the effects of active pond management on macrophyte and invertebrate diversity, which was not connected with this thesis. Within this extensive study of 45 ponds, seven ponds were recorded to have contained *C. helmsii* between 2009 and 2013. From these ponds, data had been collected on the water pH, nitrate concentration, total phosphorus concentration, and *C. helmsii* abundance (DAFOR).

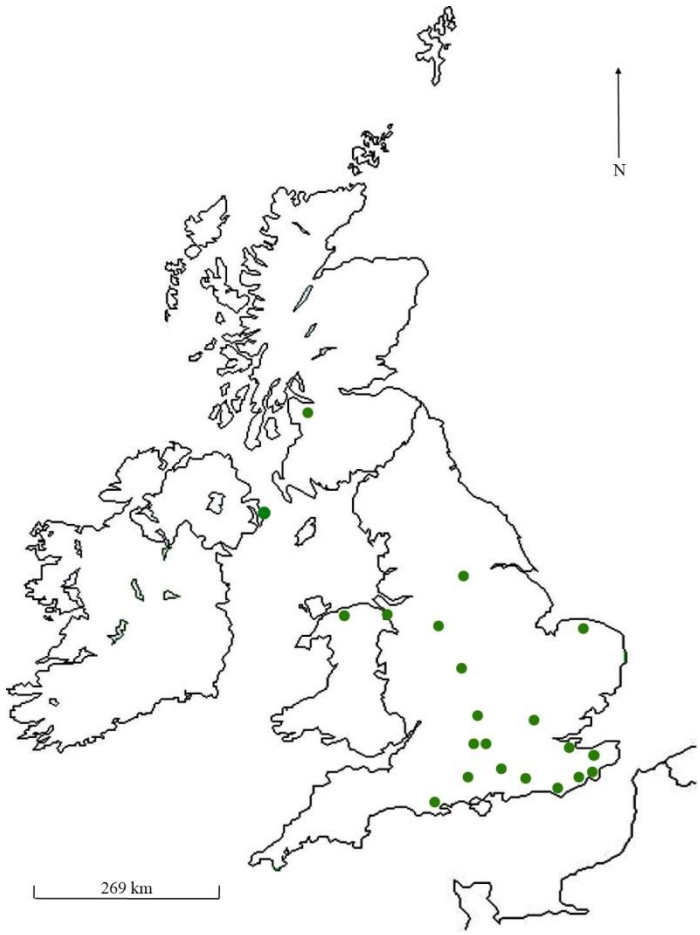


Figure 7. The green markers indicate the geographical distribution of locations, from which additional pH and macronutrient (available nitrogen and total phosphorus) data were obtained, in order to compile the regional dataset of *Crassula hemsii* invaded sites.

3.2.4. Data analysis

In the raw dataset, between-sample variation in *C. helmsii* abundance was obscured by larger scale between-site patterns of abundance. For example whilst 50 % cover was one of the highest abundances at Fen Drayton, 50 % cover was one of the lowest abundances recorded at Castle Water. To account for this effect of site, the raw data were transformed by group mean-centring before statistical analysis. This was conducted by first finding the mean value of *C. helmsii* at each of the survey sites. Then the site mean values were subtracted from each recorded data point of abundance within their corresponding sites (Field *et al.* 2012). Thus the variables were transformed from a set of raw data points, into data points which provided a value, relative to the mean value of abundance at that site. Using group mean-centring, those values which were high relative to the average amount of *C. helmsii* at the respective site, were shown as higher values in the overall linear relationship, and vice-versa. This transformation also improved the homoscedacity and the normal distribution of the residuals in the regression model. When group mean-centring was used to transform *C. helmsii* data, the same transformation was also

conducted on the abiotic or biotic variables against which *C. helmsii* was being tested. In all cases, the group mean-centred data points were calculated in Excel 2010 (© Microsoft, 2010).

Multiple linear regression was then conducted with the group mean-centred variables, using R version 3.0.2 (© the R foundation for statistical computing, 2013). The first model which was run had *C. helmsii* abundance as the dependent variable and included all five measured abiotic variables as predictors; pH, nitrate, ammonia + ammonium, total phosphorus and shading. Based on the results of this model, a second multiple linear regression was run which included only those abiotic variables which were shown to be significant predictors, as indicated by statistically significant standardised beta coefficients. AIC (Akaike's information criterion) values were also calculated for each model, as a method of assessing goodness-of-fit whilst accounting for the number of variables included in the models (Field *et al*. 2012). The second model was compared to the first based on the proportion of explained variation and the goodness-of-fit; greater explained variation being indicated by a higher R^2, and a better goodness-of-fit being indicated by higher adjusted R^2 and lower AIC values. Linear regression was also run separately on data from each of the five survey sites, using only those predictor variables which were shown to be significant in the main regression model. This was done to determine whether some sites were having a greater influence on the linear trend shown in the main model.

Further interpretation of the model was conducted by comparing the sample of abiotic data recorded in the field survey, to the sample of abiotic data documented in the regional dataset. This provided a way of assessing how well the conclusions of the field survey could be generalised to apply to other *C. helmsii* invaded sites in the UK. Comparisons were conducted simply by producing boxplots, which showed the range of raw data values for an abiotic variable in the field survey dataset, next to the range of values for the same variable in the regional dataset. Visual inspection of the boxplots showed how similar the ranges of values were in the two datasets. The data in the regional dataset was collected on a 'per invaded site' basis, meaning per isolated waterbody. To match this in the field survey dataset, mean values were calculated per waterbody (eg. pond, lake), and these values were used as the abiotic variables instead of the 94 individual sample locations.

3.3. Results

Within the field survey data, the pH ranged from 5.4 to 9.7 (mean 'M' = 7.5, standard deviation 'SD' = 1.0), nitrate ranged from below detectable levels to 10.2 mg / L (M = 1.8, SD = 2.0), ammonia + ammonium ranged from 0.012 to 8.840 mg / L (M = 0.610, SD = 1.235), total phosphorus ranged from 0.129 to 1.962 mg / L (M = 0.213, SD = 0.208), average depth ranged from 0 to 37.5 cm (M = 12.5, SD = 9.1), and shading ranged from 0 to 100 % (M = 30, SD = 40). Overall, the abundance of *C. helmsii* ranged from 0 % to 100 % cover (M = 39, SD = 37) (Fig. 8).

Multiple linear regression of the field survey data produced statistically significant models (Table 3). When all measured abiotic variables were included in the regression, the variables pH (standardised $\beta = 0.264$, $p = 0.009$) and shading (standardised $\beta = -0.397$, $p < 0.001$) were found to be significant predictors of *C. helmsii* abundance. A model which included just pH and shading as predictors, had an equivalent R^2 fit to the data and showed improved adjusted R^2 and AIC values (Table 3). This model showed a significant positive relationship between pH and *C. helmsii* abundance (standardised $\beta = 0.255$, $p = 0.008$), and a significant negative relationship between shading and *C. helmsii* abundance (standardised $\beta = -0.389$, $p < 0.001$).

When data from each of the five field survey sites were analysed separately, the strongest positive relationship between *C. helmsii* abundance and pH was shown in data from the New Forest ponds. There was also a positive relationship between *C. helmsii* abundance and pH at Ouse Fen, but a negative relationship at Castle Water, Fen Drayton, and Kingfishers Bridge. The strongest negative relationship between *C. helmsii* abundance and shading was shown in data from Kingfishers Bridge, but *C. helmsii* abundance was negatively related to shade at all sites (Table 4).

Table 3. The output from linear regression models of *Crassula helmsii* abundance predicted by a number of abiotic habitat variables. These variables were: water pH, nitrate (NO_3), ammonia + ammonium (NH_3 / NH_4^+), total phosphorus (TP), water depth, shading. The first model included all six of these variables. The subsequent three models included only pH and/or shading, as these two variables were shown to be statistically significant predictors of *C. helmsii* abundance in the first model.

Dependent	Predictors	R^2	F Value	df	p	Adj R^2	AIC
C. helmsii	pH, NO_3, NH_3 / NH_4^+, TP, depth, shading	0.220	4.006	6, 85	0.001	0.165	878.038
C. helmsii	pH, shading	0.215	12.220	2, 89	< 0.001	0.198	870.629
C. helmsii	pH	0.064	6.189	1, 90	0.015	0.054	884.830
C. helmsii	shading	0.150	15.910	1, 90	< 0.001	0.141	875.971

Table 4. The output from linear regression models of *Crassula helmsii* abundance predicted by pH and shading. Five regression models were run using data from five different *C. helmsii* invaded sites: Castle Water, East Sussex; Fen Drayton, Cambridgeshire; Kingfishers Bridge, Cambridgeshire; The New Forest (ponds), Hampshire; and Ouse Fen, Cambridgeshire.

Site	R^2	F Value	df	p	Predictor	Standardised β	p
Castle Water	0.279	2.123	2, 11	0.166	pH	-0.003	0.991
					shading	-0.527	0.081
Fen Drayton	0.065	0.657	2, 19	0.530	pH	-0.224	0.340
					shading	-0.187	0.423
Kingfishers Bridge	0.849	16.840	2, 6	0.003	pH	-0.145	0.425
					shading	-0.963	0.001
New Forest ponds	0.391	8.344	2, 26	0.002	pH	0.576	0.001
					shading	-0.366	0.027
Ouse Fen	0.194	1.923	2, 16	0.179	pH	0.192	0.406
					shading	-0.388	0.104

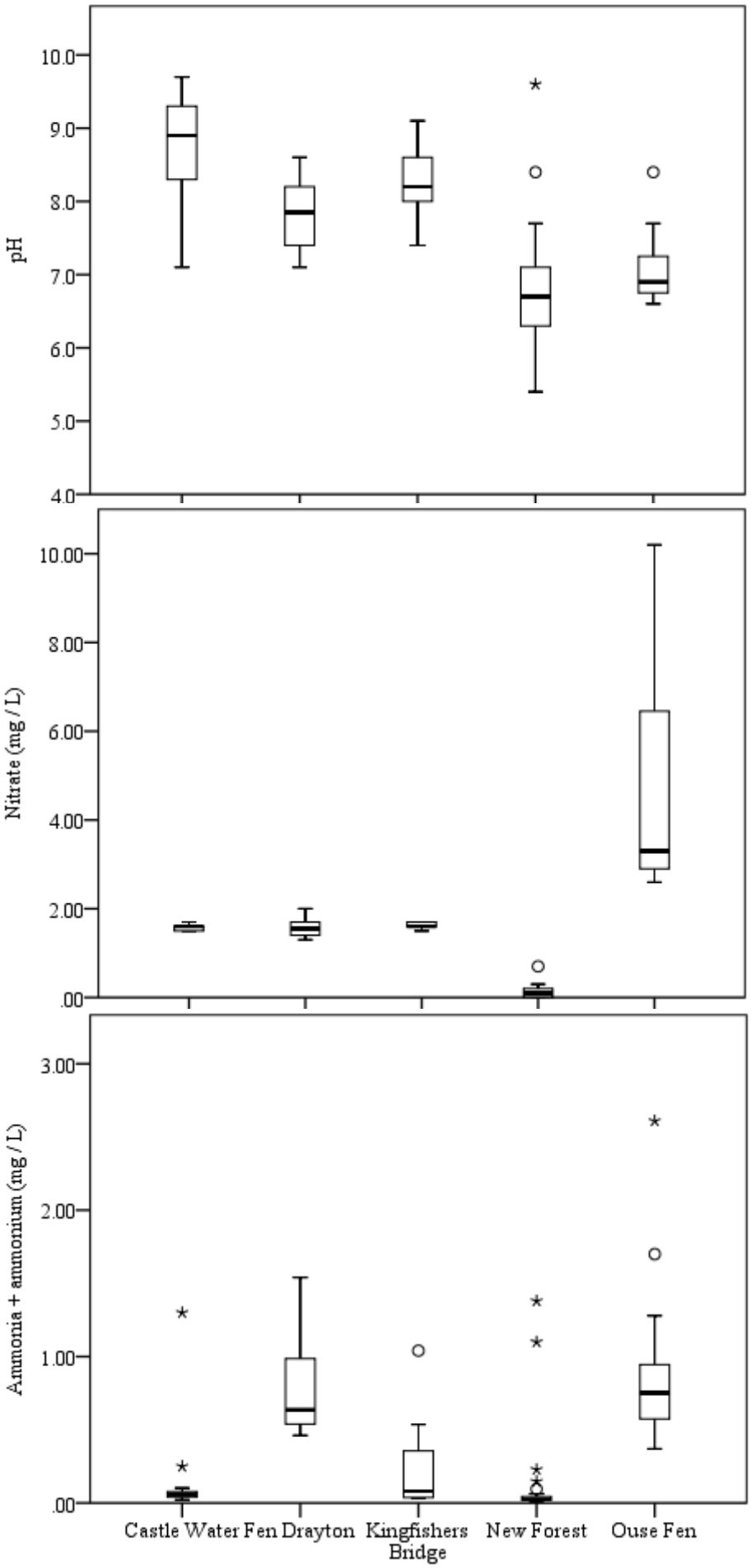
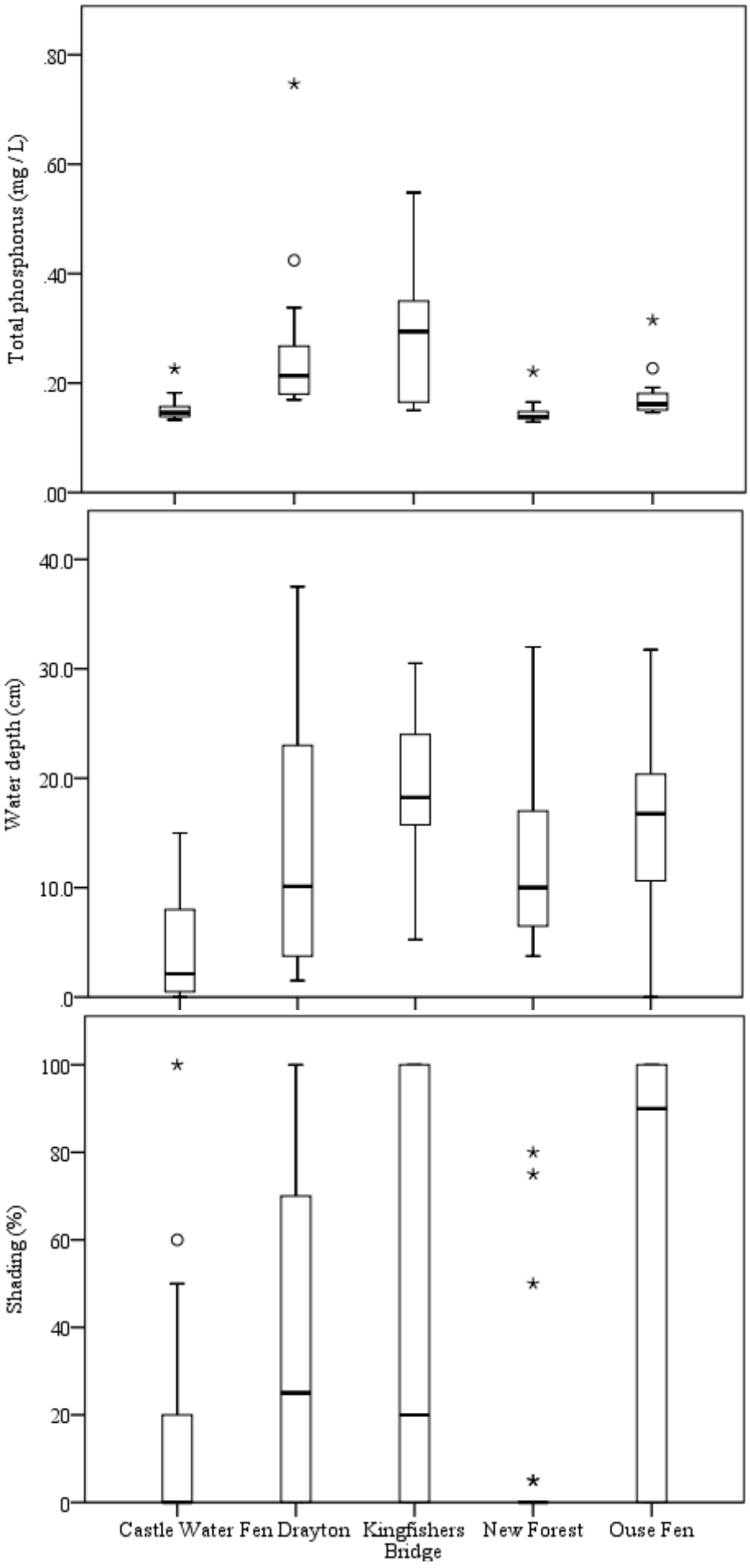


Figure 8. Continued on next two pages.



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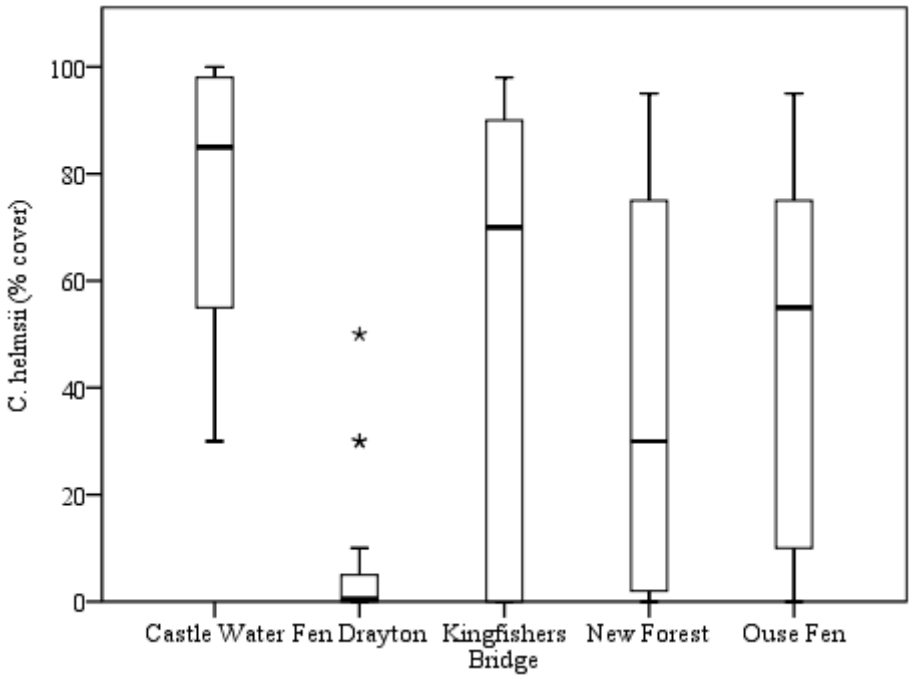


Figure 8 (and previous two pages). Each of the seven graphs relates to a measured variable. From the top of p.51: water pH, nitrate, ammonia + ammonium, total phosphorus, water depth, shading, and *Crassula helmsii* abundance. The five box plots in each graph indicate the data range for each variable, in each of the five sites that were surveyed for this study. Extreme outliers have been excluded from these graphs in order to maintain legibility. These outliers are 7.52 and 8.84 mg / L ammonia + ammonium, and 1.96 mg / L total phosphorus, all recorded at Fen Drayton.

In the regional dataset, the pH ranged from 6.0 to 10.8 (M = 7.5, SD = 0.9), nitrate ranged from below detectable levels to 83.2 mg / L (M = 6.88, SD = 15.4), ammonia + ammonium ranged from 0.289 to 2.200 mg / L (M = 0.977, SD = 0.533), and total phosphorus ranged from below detectable levels to 0.379 mg / L (M = 0.090, SD = 0.103). Of the *C. helmsii* abundance categories, at 4 sites it grew in 'dense monospecific carpets', at 22 sites it was 'highly abundant', at 11 sites it was 'fairly abundant', and at 3 sites it grew in 'infrequent patches'.

Variation in pH overlapped and was equivalent in the field survey and regional datasets. Variation in nitrates was greater in the regional dataset, encompassing more samples with higher nitrate levels. Variation in ammonia + ammonium overlapped between the two datasets, but the regional dataset contained more samples with higher ammonia + ammonium levels, and the field survey dataset contained more samples with lower ammonia + ammonium levels. Total phosphorus overlapped between the two datasets, but there was more variation in the regional dataset, which contained more samples with higher and with lower total phosphorus levels (Fig. 9).

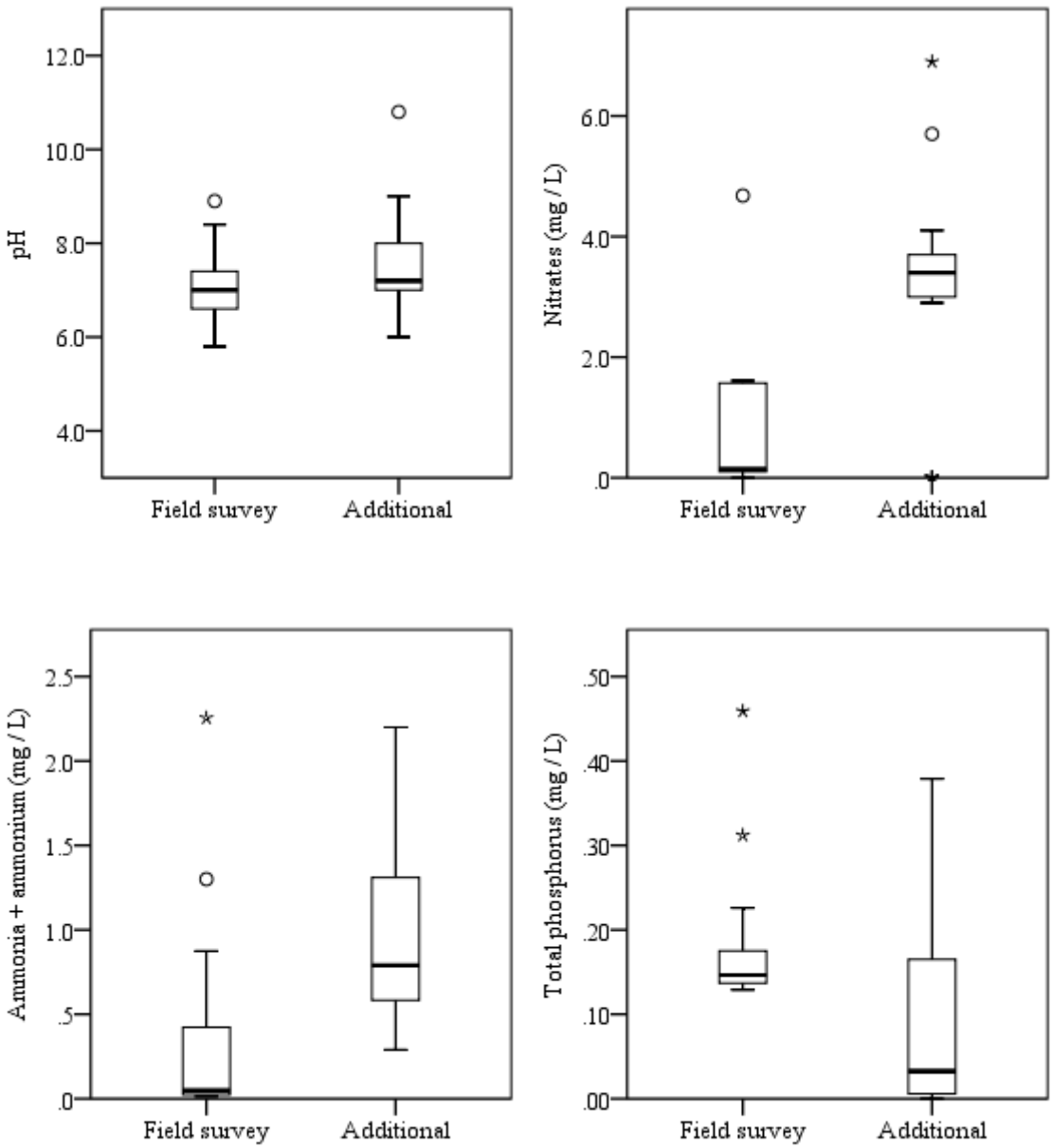


Figure 9. A visual comparison of the range of pH and macronutrient concentrations (nitrate, ammonia + ammonium, total phosphorus) measured in the 'field survey' dataset, to the range of the same measured variables in the 'additional' regional dataset. The 'field survey' data were collected from water bodies in Southern and Eastern England, whereas the 'additional' regional data were obtained from waterbodies across a wider UK distribution. It can be seen that the range of pH was roughly equivalent between the two datasets, but that the 'field survey' dataset had a lower range of available nitrogen, and a smaller range of total phosphorus. Extreme outliers of 83.2, 24.8, and 13.4 mg / L nitrate, recorded in the 'additional' regional dataset, have been excluded from the graph in order to maintain legibility.

3.4. Discussion

The abundance of *C. helmsii* in the field was positively associated with pH and negatively associated with the amount of shading, but macronutrient levels and the depth of water in the marginal zone had little influence on *C. helmsii* abundance. The best-fit linear regression model indicated that variation in pH and shading accounted for 21.5 % of the variation in *C. helmsii* abundance, with shading accounting for more of this explained variation than pH. Accordingly there was a considerable amount of unexplained variation in the model, and so there are likely to be a number of factors not included in this survey which affected *C. helmsii* abundance. Most obviously abundance could have varied due to the strength of interspecific competition imposed by the resident plant community (Levine *et al.* 2004; Mitchell *et al*. 2006), with *C. helmsii* becoming more abundant were there was less competitive pressure. This topic is addressed in more detail in chapter seven. The amount of time elapsed since colonisation of an area (Theoharides & Dukes, 2007) could also have accounted for a proportion of the unexplained variation in abundance. Nevertheless based on this study, knowledge of pH and shading levels represent a useful contribution to site information when making predictions of *C. helmsii* abundance in the field.

An overall significant positive relationship was found between *C. helmsii* abundance and pH, suggesting that *C. helmsii* abundance was relatively high, where the pH was relatively high. Importantly however, inspection of this relationship when data were tested separately per site, suggests that the quadrat samples from the New Forest ponds may have had a disproportionally large effect on the outcome of the main linear model. The overall range of pH measured in the field survey was pH 5.4 to 9.7 however nearly all quadrats with an acidic pH were located in the New Forest ponds. Consequently, data from the New Forest ponds had a disproportionally large effect on the outcome of the main linear model, because *C. helmsii* abundance was only being limited by pH in acidic conditions. Significantly, the quadrat location with the lowest measured pH (5.4) did not contain any *C. helmsii*, although this species was found growing in the quadrat location with the next lowest pH (5.8). In other sites, a significant relationship with pH may not have occurred because *C. helmsii* abundance was not being limited by pH within the neutral to alkaline range. The distribution of the pH sample in this survey further suggests that *C. helmsii* may be more limited in acidic conditions; 30 % of quadrats measured had a pH lower than 7.0, and only 4 % had a pH lower than 6.0. Whilst it is possible that this distribution may simply have been reflective of the pH within the chosen sample sites, the fact that the same pH distribution was found in the regional dataset sample, adds confidence to the generalisation that *C. helmsii* more typically occurs in sites with a neutral to alkaline pH.

Further work is required to test the hypothesis that *C. helmsii* abundance was limited in an acidic pH, but not in a neutral or alkaline pH. Firstly, it would be beneficial to extend data

collection to encompass more sites where the range of pH is between neutral and acidic, to determine whether the same positive relationship is found in locations other than the New Forest ponds. Secondly, experiments could be conducted which assess the growth rate of *C. helmsii* under controlled conditions of acidic, neutral, and alkaline pH. Such growth trials were attempted for this thesis however the pH was not successfully controlled (appendix II).

An overall significant negative relationship was found between *C. helmsii* abundance and shade, and a negative relationship was also found in all sites when data were tested separately per site. This suggests that *C. helmsii* abundance was relatively high where shade levels were relatively low. In particular at Kingfishers Bridge, shading was found to be a significant predictor of a large proportion of variation in *C. helmsii* abundance. Concurrently the distribution of *C. helmsii* at this site was distinct; *C. helmsii* occurred in high abundance along the edges of the lake where there was open draw down zone, and was absent from the edges where there were dense *Phragmites autralis* reedbeds shading the drawdown zone. Importantly this demonstrates that in this survey, the consistent source of shading was taller, overtopping vegetation. *Crassula helmsii* was not totally excluded from shady habitat however, and especially in quadrats located at Ouse Fen this species was found growing underneath dense stands of *P. australis*. As discussed in the introduction to this chapter, *C. helmsii* has been documented as tolerant of shade (Hill *et al*. 2004), and Hussner (2009) reported that this species had lower light requirements than three other invasive aquatic plant species. It is likely therefore, that unshaded habitats represent optimal conditions for *C. helmsii* in the UK, but that this species can persist in shady habitats, although at lower abundances. Growth experiments with controlled levels of shade, for example using shade net of varying opacity (Skálová *et al*. 2013), could be used to improve confidence in this hypothesis.

In this study macronutrient levels were not found to be significant predictors of *C. helmsii* abundance. This result seems contradictory to laboratory findings that *C. helmsii* growth rate increases with nutrient availability (Hussner, 2009; appendix II), and descriptions of *C. helmsii* as typical of higher nutrient level conditions (Hill *et al*. 2004; Minchin, 2008). One reason why this relationship was not detected could be that *C. helmsii* faced stronger competition from co-occurring plants in the locations where nutrients were highest. Indeed, increased community resistance to invasive plant dominance, has been recorded in response to increased nitrogen levels, in grassland plant communities (Going *et al.* 2009). Alternatively, it is possible that nutrient levels do influence the abundance of this species, but that the relationship was not detected in this field survey because the nutrient level sample did not encompass enough variation. To support this suggestion, the regional dataset indicated that *C. helmsii* could occur across a much wider range of nutrient levels than was included in the main field survey, and especially in conditions with higher nitrogen levels. Similarly, whilst water depth was not found to be a significant predictor of *C. helmsii* abundance in water of 0 to 38 cm, it is possible that a

relationship could have been detected if the survey methodology had allowed for sampling at greater depths.

This chapter was designed to provide information on whether simple abiotic variables, could be used to reliably predict where high *C. helmsii* abundance was likely to occur, and in this way assess how important the abiotic habitat was in facilitating the growth of dense, dominating *C. helmsii*. Based on the results of this survey, it seems that high *C. helmsii* abundance is more likely in wetland habitats where the pH is neutral to alkaline, and in open sites which lack tall, shading vegetation.

NB. The following chapter was published as a paper in the journal Conservation Evidence, on 11th December 2013:

Dean C., Day J., Gozlan R.E., Green I., Yates B. Diaz A. 2013. Estimating the minimum salinity level for the control of New Zealand Pygmyweed *Crassula helmsii* in brackish water habitats. *Conservation Evidence*. **10**. 89-92.

The format of this data chapter therefore follows the style of this journal.

4. What is the estimated minimum salinity level for the control of *Crassula helmsii* in brackish water habitats?

4.1. Background

The non-native semi-aquatic plant New Zealand Pygmyweed *Crassula helmsii* has invaded and become abundant within many nature reserves across Europe where land is managed as wetland habitat such as ponds, shallow pools, reedbeds, and grazing marsh (Langdon *et al.* 2004; Bridge, 2005; Gomes, 2005; Wilton-Jones, 2005). *Crassula helmsii* can spread rapidly across bare mud (personal observation), and heavy invasions resemble thick green carpets, with few other plant species occurring in amongst the dense vegetation (Dawson & Warman, 1987; EPPO, 2007; Minchin, 2008). Degrading the invasive vegetation using herbicides, heating, or covering with light eliminating material, can decrease the abundance of *C. helmsii* but complete eradication is rarely achieved due to subsequent re-growth of remaining vegetative fragments (Bridge, 2005; Gomes, 2005; Wilton-Jones, 2005).

The use of seawater inundation has been found to be a practical option for the control of *C. helmsii* in coastal habitats. For example, Charlton *et al.* (2010) found that *C. helmsii* was eradicated from grazing marsh at RSPB Old Hall Marshes by flooding the area with seawater. At this site, the benefit of *C. helmsii* eradication was carefully weighed against the potential negative impacts on co-occurring native species (Charlton *et al.* 2010; Gardiner & Charlton, 2012). Such considerations are important as saltwater inundation is a non-targeted method of control, so any other organisms occurring within an inundation zone would also experience a sudden rise in salinity; those which cannot tolerate the rise in salinity would also be locally eradicated. Reviews of invasive plant control efficacy have also highlighted that the costs to native species is an important consideration when planning best practice control attempts (Kettenring & Adams, 2011).

There is limited published information on salinity tolerance in *C. helmsii*. Whilst it can be deduced that this invasive plant has some tolerance to saline water due to its invasion of coastal habitats (EPPO, 2007), this species is not a brackish habitat specialist and its UK distribution is more associated with freshwater (Preston & Croft, 1997). Knowledge of the environmental tolerances of an invasive species can be used to create conditions which are less favourable for the invader (Davis, 2009). In the current context knowing the threshold between freshwater and saline water at which *C. helmsii* can no longer grow, allows manipulation of site salinity to be used as a management tool. Furthermore, such information has utility when predicting how the

abundance and distribution of the invasive plant may change, if land management is altered in a way which affects the salinity of a site (Thouvenot *et al.* 2012).

The aim of this investigation was to determine the levels of salinity required to kill *C. helmsii*. In doing so we aimed to provide information on the increase in salinity needed at a site in order to eradicate *C. helmsii,* whilst minimising saline toxicity in co-occurring native species. We conducted growth trials with the objective of finding the lowest level of salinity which kills *C. helmsii*. The concentration of plant available nutrients was also controlled, as nutrient availability in tank experiments has previously been found to affect the growth rate of *C. helmsii* (Hussner, 2009).

4.2. Action

The growth trials were conducted outdoors at Bournemouth University, Dorset, UK (OS grid ref: SZ 073 937). *Crassula helmsii* was grown in conditions specifically modified to produce three different levels of salinity; 2, 4, and 8 parts/thousand ('ppt'). In contrast seawater has a salinity of greater than 30 ppt. The three test levels of 2, 4 and 8 ppt were chosen based on field observations of the distribution of *C. helmsii* in brackish water pools at Pett Level in East Sussex (OS grid ref: TQ 903 147), where this plant was restricted to water of 4 ppt and below.

To set up the growth trials, we used 5 L plastic storage containers, lined with horticultural sand to 3 cm depth and filled with 1 L of distilled water, to mimic shallow water habitat conditions suitable for *C. helmsii*. Fresh cut samples of *C. helmsii* weighing 10 g were added to each 5 L container. The correct salinity was achieved at the start of the experiment by mixing 2, 4, or 8 g of salt (sodium chloride) to the 1 L of distilled water. A solution containing all required mineral elements for growth (Taiz & Zeiger, 2006), was applied to the water + salt solutions at the start of the experiment. The nutrient solution was applied at three different dilutions; 0.125, 0.25 and 0.5 times the full strength solution. Each salinity level was combined with each nutrient dilution, making nine different salinity/nutrient treatments, and each treatment was replicated four times in a separate container. To act as a control, additional containers were set up in which 10 g fresh weight samples of *C. helmsii* were grown within the same three nutrient dilutions but with no added salt, replicated three times each. In total therefore, *C. helmsii* was grown in 45 separate containers.

The experiment lasted for 31 days, during September and October 2011. Met Office records of climatic data from Hurn weather station (approximately 4 miles north-east of the trial location) report that in September 2011 daily temperature averages ranged from 19.8 °C to 11.3 °C, with 0 days air frost, and in October 2011 daily temperature averages ranged from 17.3 °C to 8.6 °C, with 1 day air frost (© Crown copyright, 2011). At the end of the experiment, the *C. helmsii* was removed from each container and thoroughly rinsed with tap water. In order to obtain a dry weight value for the amount of *C. helmsii* growing in each container at the end of the experiment, the vegetation was dried at 90 ˚C for 48 hrs before being weighed. A dry weight value for the start of the experiment was estimated by weighing out twenty additional *C. helmsii* samples of 10 g fresh weight, drying them at 90 ˚C for 48 hours, and taking the average dry weight of these samples. Having the average 'start dry weight', and the 'end dry weight' for each *C. helmsii* sample allowed for the relative growth rate to be calculated for each replicate.

This was done using the formula:

$$\text{Relative growth rate} = \ln(W2) - \ln(W1) / (t2 - t1)$$

Where W1 is the start and W2 the end dry weight, ln is the natural logarithm, and t1 is the start and t2 the end time measured in days. The formula describes the proportional increase of a plant in grams, expressed per gram per day ($g\ g^{-1}/day^{-1}$) (Hunt, 2003).

4.3. Consequences

A significant difference was found in average growth rate between the salinity treatments (two-way ANOVA: $F_{(3, 33)} = 197.705$, $p < 0.001$) (Fig. 10). The highest average growth rates were recorded within the control (0 ppt) (Table 5). Growth was recorded at 4 ppt but the average growth rates were lower than at 2 ppt or in the control. In the 8 ppt treatments, all but one replicate was found to have a negative growth rate which is indicative of a loss of biomass. By the end of the experiment it was observed that the *C. helmsii* within the 8 ppt salinity treatments had died and was in the early stages of decomposition. The nutrient dilution had no significant effect on the growth rate of *C. helmsii* in this experiment (two-way ANOVA: $F_{(2, 33)} = 0.115$, $p = 0.892$).

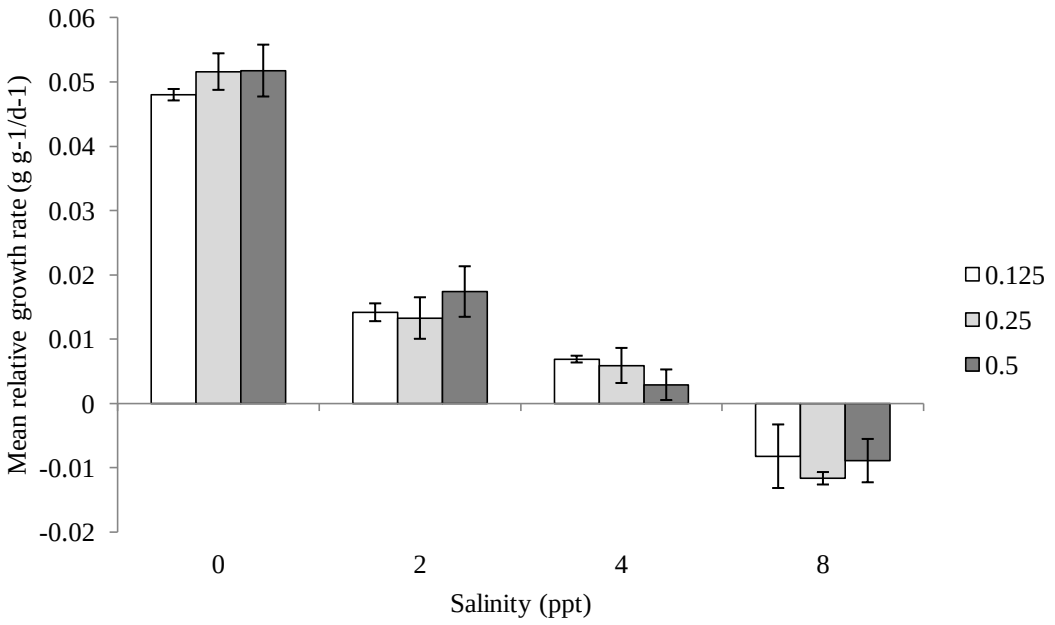


Figure 10. Bar charts showing the relative growth rate (± 1 SE) of *Crassula helmsii* when grown in tanks, with a factorial design combining three treatment levels of salinity (2, 4, and 8 parts/thousand) plus a control, and three treatment levels of nutrient dilution (0.5, 0.25, and 0.125 x full strength nutrient solution). Bars falling below the 0.00 line represent negative growth, and thus biomass loss.

Table 5. The average start dry weight, end dry weight, and growth rate values for replicate trials of *Crassula helmsii* when exposed to three different levels of salinity (2, 4, and 8 parts/thousand) plus a control, combined with three different nutrient dilutions (0.5, 0.25, and 0.125 x full strength nutrient solution).

Salinity (ppt)	Nutrient dilution (x full strength)	Average values		
		Start dry weight (g)	End dry weight (g)	Growth Rate ($g\ g^{-1}/day^{-1}$)
0	0.125	0.840	2.790	0.048
0	0.25	0.840	3.067	0.052
0	0.5	0.840	3.093	0.052
2	0.125	0.840	1.288	0.014
2	0.25	0.840	1.268	0.013
2	0.5	0.840	1.445	0.017
4	0.125	0.840	1.033	0.007
4	0.25	0.840	1.013	0.006
4	0.5	0.840	0.923	0.003
8	0.125	0.840	0.678	-0.008
8	0.25	0.840	0.593	-0.012
8	0.5	0.840	0.653	-0.009

4.4. Discussion

The results of the growth trials show a negative linear relationship between *C. helmsii* growth and salinity, indicating that *C. helmsii* growth is inhibited by increasing salinity across the range from 2 to 8 ppt. Importantly *C. helmsii* died in tanks of 8 ppt, suggesting that this is beyond the salinity tolerance of this species. Thus, based on the findings of these growth trials, we suggest that if the salinity of a *C. helmsii* invaded site were raised to 8 ppt or above, this could have similar eradication efficacy to that produced by seawater of 30 ppt. Furthermore, if a site could be permanently maintained at 8 ppt or higher, this could provide a long-term solution for the prevention of re-invasion in sites where *C. helmsii* has been eradicated, or prevent its spread within habitats where the species has a patchy distribution.

Using the lowest effective salinity for *C. helmsii* eradication may represent a way to minimise the impacts of saltwater inundation. In particular this method may minimise impacts in naturally brackish habitats, where an increase to 8 ppt may be enough to inhibit *C. helmsii* whilst favouring native brackish habitat specialists. For example plant species such as saltmarsh goosefoot (*Chenopodium chenopodioides*), brackish water-crowfoot (*Rununculus baudotii*), sea clubrush (*Scirpus maritimus*) and greater sea-spurrey (*Spergularia media*) have all been recorded as co-occurring with *C. helmsii* at Pett Level in East Sussex. In addition, the salt tolerance of common reed (*Phragmites australis*) (Preston & Croft, 1997; Chambers *et al.* 2003) may make brackish water (8 ppt) inundation an appropriate method of *C. helmsii* eradication in mature reedbed systems.

This paper represents a demonstration of concept. We acknowledge that the next step will be to test whether *C. helmsii* is successfully eradicated in the field using inundation with water at 8 ppt, and whether control of salinity levels is a feasible management option. Additional tank-based growth trials could also be conducted to estimate the minimum length of time inundated at 8 ppt, which is required to ensure a total kill. We recommend that field trials be conducted in sites where the predominant co-occurring plant community are tolerant of brackish conditions. Furthermore, monitoring of the abundance of co-occurring native species in such field trials, would provide evidence of whether *C. helmsii* is more greatly inhibited at 8 ppt than plants which are brackish habitat specialists.

NB. The following chapter was re-written as a research article and published in the journal Invasive Plant Science and Management:

Dean, C.E. Day, J. Gozlan, R.E. Diaz, A. 2015. Grazing vertebrates promote invasive swamp stonecrop (*Crassula helmsii*) abundance. *Invasive Plant Science and Management*. In Press. Available from: http://www.wssajournals.org/doi/10.1614/IPSM-D-14-00068.1.

5. What is the effect of vertebrate grazing disturbance on the abundance of *C. helmsii* within a wetland plant community?

5.1. Introduction

The effect of grazing disturbance on plant community structure is variable, dependent on the grazing intensity, the type of grazing animal, and the individualistic response of plant species to this form of disturbance (Milchunas *et al*. 1988; Olff & Ritchie, 1998; Bullock *et al*. 2001; Bakker *et al*. 2006). However, it is generally recognised that herbivory can directly affect the composition of plant communities by altering the survival and biomass of individual species, and can indirectly affect the composition by altering plant-plant interactions (Crawley, 1997; Olff & Ritchie, 1998; Huston, 2004; Rook *et al*. 2004; Jones *et al*. 2011). It is therefore possible that grazing disturbance can influence the extent to which a non-native invasive plant produces dominating invasive growth, both by directly affecting the invader, and by mediating the abundance and competitive strength of co-occurring plant species. Predicting how grazing might influence a non-native invasive plant requires an understanding of how different forms of grazing affect the non-native invasive species, relative to how they affect other plant species in the invaded range (Mitchell *et al*. 2006).

In situations where non-native invasive plants show high productivity (Davis, 2009; Vilà *et al*. 2011) this competitive growth may encourage grazing (Olff & Ritche, 1998). In particular non-native invasive species may be subject to attack from generalist herbivores (Maron & Vilà, 2001), indeed experiments using invertebrate herbivores have shown that some non-native invasive species can be equally or more palatable to generalists than native plants (Agrawal & Kotanen, 2003; Parker & Hay, 2005). Such attack may therefore suppress the abundance of a non-native invasive plant, and furthermore, removal of seedlings may limit establishment (Maron & Vilà, 2001; Stricker & Stiling, 2012; Fan *et al*. 2013).

In contrast, grazing could favour the growth and dominance of a non-native invasive species. For example if stronger competitors were reduced by grazers, then the non-native invasive species could benefit from greater access to light, space and nutrient resources (Xie *et al*. 2013). Furthermore, suppression of native seedlings could prevent stronger competitors from establishing, promoting the persistence of the non-native invasive plant (Baiser *et al.* 2008). The 'enemy release hypothesis' suggests that invasive growth of a non-native species can arise if the plants are selected less, or selected by fewer grazing species. Specifically this hypothesis suggests that non-native invasive species experience reduced herbivory in an introduced range, due to the absence of specific herbivores which have co-evolved with the plant in its native

range (Tilman, 1999). Summative evidence for the relative strength of this mechanism is however still lacking (Keane & Crawley, 2002; Davis, 2009). An alternative hypothesis is that non-native invasive species experience similar levels of grazing as the natives due to attack from generalist herbivores, but increase in abundance because they are less negatively affected, or respond more positively to this kind of disturbance (Kimball & Schiffman, 2003; HilleRisLambers *et al.* 2010; Stahlherber & D' Antonio, 2013).

Because *C. helmsii* occurs in habitats which use livestock grazing as a management tool; for example fens and grazing meadows (Preston & Croft, 1997); it is important to investigate how this non-native invasive plant responds to disturbance created by large vertebrates. In particular, does the presence of grazing livestock influence whether *C. helmsii* forms dense dominating vegetative mats? Light grazing by livestock, such as cattle and sheep, is used in the management of fen habitat to encourage plant species diversity, by suppressing the abundance of tall, highly competitive and dominating plant species. This suppression may occur both via consumption of biomass, and via vegetative damage from trampling (Natural England, 2005; McBride *et al.* 2011). As a plant species which can produce extensive above-ground biomass, it may be hypothesised that the presence of grazing animals could limit the overall abundance of *C. helmsii*, through consumption or trampling degradation. Grazing could therefore, offer a way of limiting the dominance of this invasive species in fen habitat. However, this hypothesis is predicated on the assumption that *C. helmsii* would be selected by grazing animals or that it would be suppressed by trampling, and furthermore, that it would experience more negative impacts from grazing disturbance than other plant species in the community.

The aim of this study was to test whether the presence of grazing disturbance affected the relative abundance of *C. helmsii* within a plant community, and further whether grazing had a suppressive or facilitative effect on *C. helmsii* abundance. The benefits of this study were thus twofold; firstly it offered insight into whether grazing disturbance is an ecological factor which can mediate dominating invasive *C. helmsii* growth, and secondly it offered an assessment of whether livestock grazing is an appropriate management technique for maintaining higher plant species diversity, in *C. helmsii* invaded habitats. To address this aim, a field experiment was conducted in which grazing exclosures were set up along a heavily *C. helmsii* invaded lake drawdown zone, in a fen habitat where livestock grazing was used as a management tool to limit the abundance of tall, dominating herbaceous species. The abundance of *C. hemsii* and the co-occurring plant community was monitored in these exclosures, and was compared with control plots which were accessible to large vertebrates. Three questions were asked; 1) Is there an effect of grazing disturbance on the abundance of *C. helmsii*? 2) Is there an effect of grazing disturbance on the abundance and species diversity of co-occurring vegetation? 3) Is there an effect of grazing disturbance on species dominance?

5.2. Methods and Materials

5.2.1. Experimental design

In this experiment the effect of disturbance from grazing vertberates was ascertained, by observation of changes to the plant community when these animals were excluded. The experiment was sited at Kingfishers Bridge nature reserve. For a description of the site see chapter two, section 2.1. Six grazing exclosures were set up along a wide section of the drawdown zone, on the southern margin of a shallow lake, where *C. helmsii* was growing in abundance. These exclosures were 4 m^2 in area with 1m high fencing, and designed to impede access by medium to large vertebrates. The exclosures were set up on 28th February 2012 pre-empting spring vegetation regeneration; at this stage in the year the drawdown zone was predominantly bare ground with patches of *C. helmsii* which had persisted through the winter (Fig. 11). At the same time the positions of six grazed (unfenced) 4 m^2 plots were designated, interspersed within the grazing exclosures but separated by a buffer zone of 1m, and located so that all plots experienced water levels of an equivalent depth.

Grazing pressure and livestock type varied during the experiment based on the habitat management decisions of the reserve warden: sheep were grazed from January to March 2012 and from August 2012 to October 2013, and 11 buffalo were grazed from late July to December 2012. The site was also frequently visited throughout the year by herbivorous wildfowl. This variation meant that *C. helmsii* response to grazing intensity could not be ascertained, and this experiment purely focuses on the response of *C. helmsii* to the presence or absence of disturbance from vertebrate grazing.

The plots were surveyed eight times between July 2012 and October 2013, with a two month gap between each subsequent survey. The first survey was conducted on 16th July 2012 to coincide with the end of the ground nesting bird season and when seasonal vegetation growth was present. The final survey was conducted on 4th October 2013 to coincide with the end of the main growing season. In each plot the abundance of *C. helmsii* was recorded as an estimate of percentage cover. All co-occurring plants were identified to species using standard keys for vascular plants (Rose, 1989; Rose, 2006), and the percentage cover was estimated separately for each species. The maximum height of vegetation was recorded throughout the experiment, and the maximum height of *C. helmsii* specifically was recorded during the last four surveys of the experiment, as an added line of enquiry.

Figure 11. Photograph showing the 4m^2 grazing exclosures during the set up of the experiment in February 2012. These exclosures, along with paired unfenced plots, were situated along a lake drawdown zone at Kingfishers Bridge nature reserve, Cambridgeshire, where *Crassula helmsii* was known to have invaded (© Roger Beecroft).

5.2.2. Statistical analysis

Is there an effect of grazing disturbance on the abundance of C. helmsii?

The effect of grazing disturbance on *C. helmsii* abundance was assessed with a linear mixed effects model, using the R package 'nlme' (Pinheiro *et al.* 2013) in R version 3.0.2 (© the R foundation for statistical computing, 2013). Before analysis percentage cover data for *C. helmsii* were arcsine transformed to normalise the data (see chapter two, section 2.2). Then data from all eight surveys were included in a linear model, to test for an effect of 'grazing treatment' on *C. helmsii* abundance. The variable 'survey' was included as a random factor in this model in order to account for the non-independence of data collected from the same plots over the 8 survey dates.

Is there an effect of grazing disturbance on the abundance and species diversity of co-occurring vegetation?

The abundance of co-occurring vegetation was calculated as the sum of the cover of all plant species recorded within a plot on a particular survey date, excluding *C. helmsii*. All vegetation percentage cover data were arcsine transformed before analysis. To quantify plant species diversity in each plot, the Shannon-Weiner diversity index (H) was calculated (see chapter two, section 2.2), and again this variable excluded *C. helmsii*. Abundance and species diversity were tested separately for an effect of grazing, using data from all eight surveys, in linear mixed effects models with 'survey' included as a random factor.

Is there an effect of grazing disturbance on species dominance?

Dominance was scored based on proportional abundance, defined as the abundance of a plant species relative to the total amount of vegetation cover recorded in a plot, and calculated using the formula;

Proportion = (% cover single species / total % cover all species)*100

The plant species with the highest proportional abundance within a plot, equated to the most dominant species. Using these data the most dominant species was determined for each plot in each survey, allowing comparisons to be made between the grazed and ungrazed treatments.

The magnitude of dominance was also assessed, by comparing the proportional abundance of the dominant species, to that of other abundant species within the same plot. In this study, species were considered 'abundant' if they were present in at least four out of the eight sequential surveys, and if they had a percentage cover of 10 % of higher in at least three out of the six plots, per treatment. Linear mixed effects models were used to statistically compare the proportional abundance of species within the same plot, through calculation of the mean difference and *t* values. These models used arcsine transformed 'proportional abundance' as the dependent variable, and 'species' as the fixed factor, with 'plot' nested within 'survey' as random factors. Data from grazed and ungrazed treatments were analysed separately.

5.3. Results

Is there an effect of grazing disturbance on the abundance of C. helmsii?

There was a significant effect of grazing treatment on *C. helmsii* abundance ($t_{(87)}$ = 28.974, p < 0.001), with mean *C. helmsii* abundance found to be lower in the ungrazed plots than in the grazed plots. The abundance of *C. helmsii* was found to decrease over time in the ungrazed plots, whilst remaining constant in the grazed plots (Fig. 12). Measurements of the maximum *C. helmsii* height suggest a trend for taller growth in the ungrazed plots during the summer months (Fig. 13).

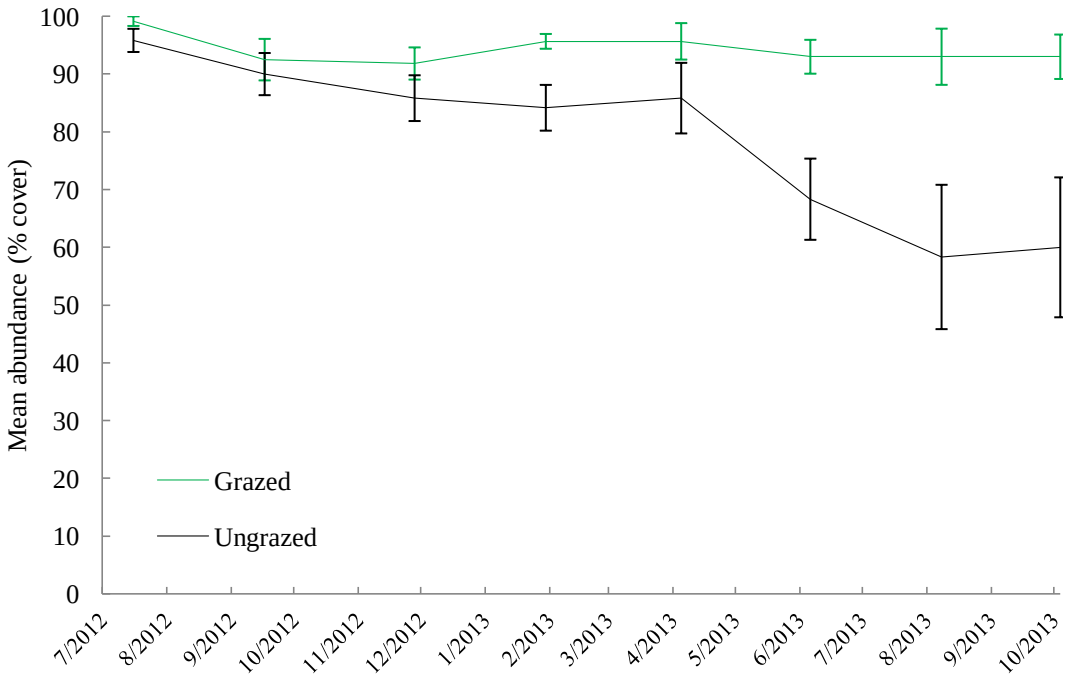


Figure 12. The mean percentage cover (± 1 SE) of *Crassula helmsii* in two separate experimental treatments: 'grazed' unfenced plots, and 'ungrazed' fenced exclosures. The graph shows the change in mean percentage cover over the course of eight repeated surveys that were carried out between July 2012 and October 2013.

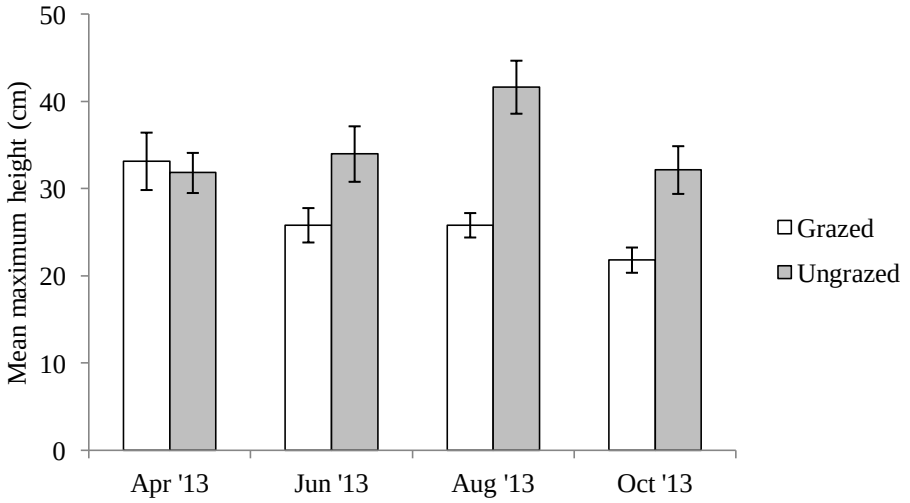


Figure 13. The mean maximum height (± 1 SE) of *Crassula helmsii* in 'grazed' unfenced plots, and 'ungrazed' fenced exclosures, in the final four of eight repeated surveys.

Is there an effect of grazing disturbance on the abundance and species diversity of co-occurring vegetation?

There was a significant effect of grazing treatment on co-occurring vegetation abundance ($t_{(87)}$ = 6.264, p < 0.001), and on co-occurring species diversity ($t_{(87)}$ = 2.647, p = 0.010). Mean abundance was consistently higher in the ungrazed plots, and mean species diversity was higher in the ungrazed plots in all but the first two surveys of the experiment (Fig. 15). The mean maximum height of vegetation was also shown to be consistently higher in the ungrazed plots (Fig. 14).

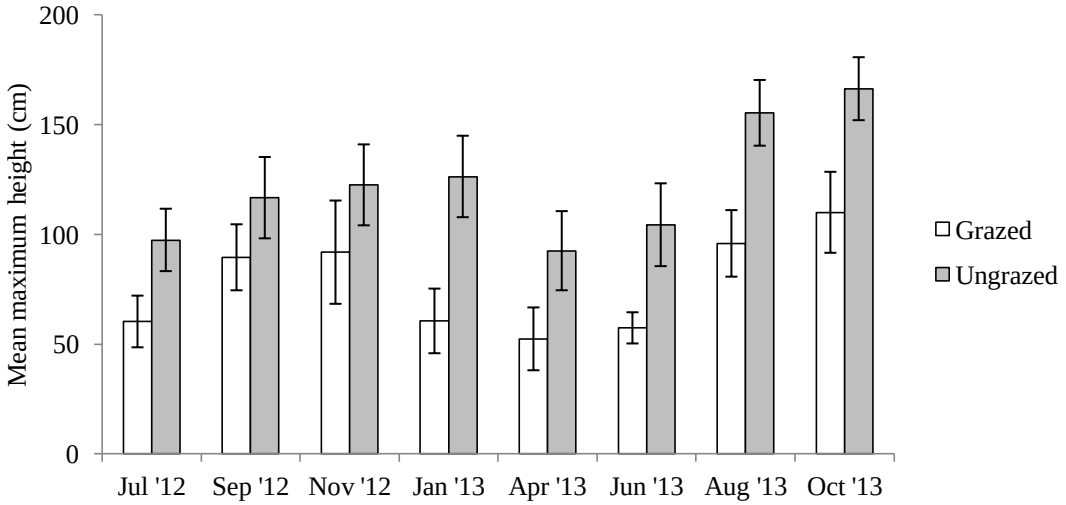


Figure 14. The mean maximum height (± 1 SE) of all vegetation, excluding *Crassula helmsii*, in 'grazed' unfenced plots, and 'ungrazed' fenced exclosures. The graph shows data collected over eight repeated surveys.

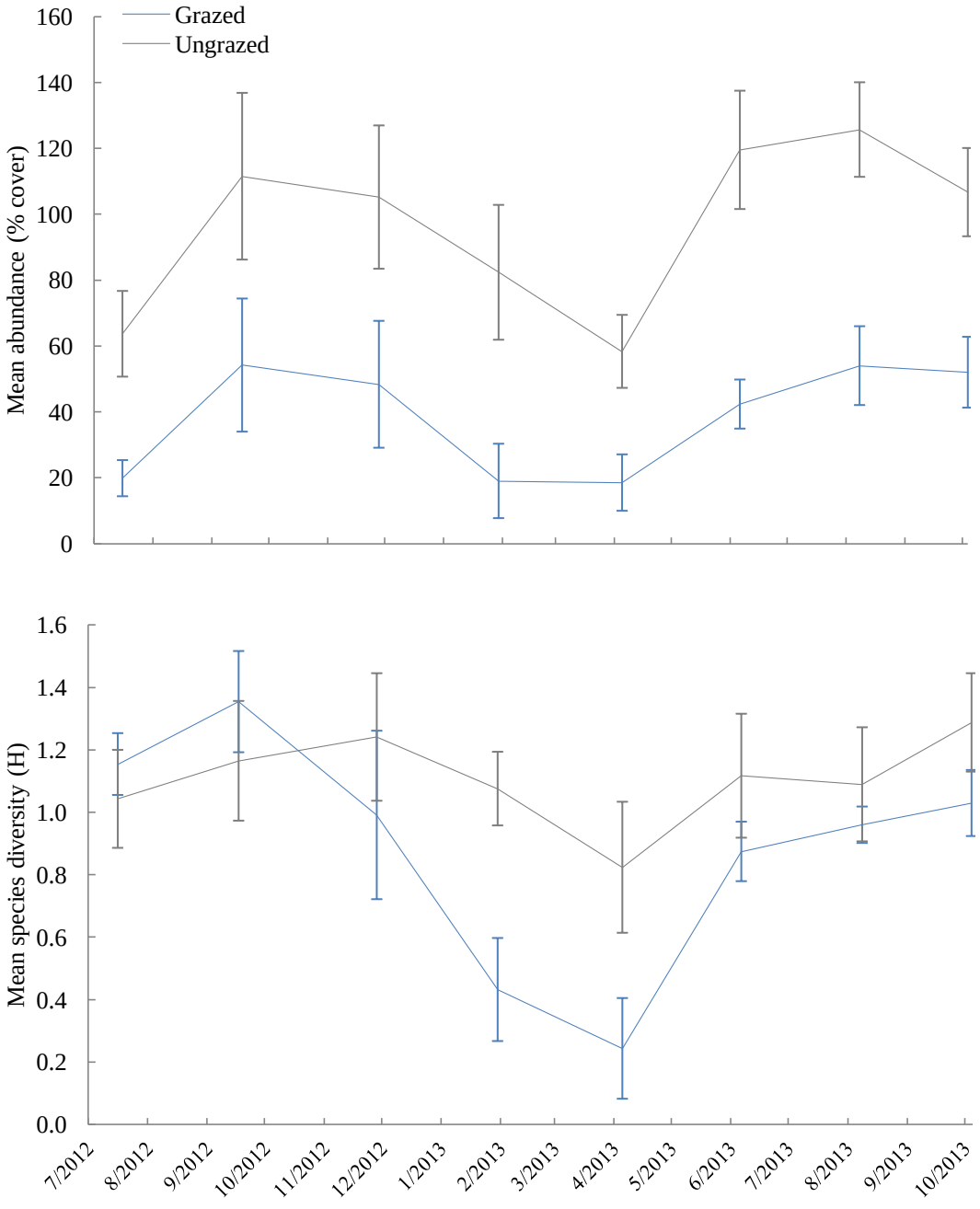


Figure 15. The top graph shows the mean percentage cover (± 1 SE) of all vegetation excluding *Crassula helmsii*, and the bottom graph shows the Shannon-Weiner H species diversity index (± 1 SE) for this vegetation, over eight repeated surveys. Both graphs indicate the change over time in these variables, in two separate experimental treatments: 'grazed' unfenced plots, and 'ungrazed' fenced exclosures.

Figure 16. Photograph taken in August 2013 showing a fenced plot (ungrazed treatment) on the left, and an unfenced plot (grazed treatment) on the right.

Is there an effect of grazing disturbance on species dominance?

Based on the criteria for abundance detailed in the methods section above, two species were classed as abundant in the grazed plot treatments (*C. helmsii* and *Mentha aquatica*) and three species were classed as abundant in the ungrazed plots (*C. helmsii*, *Eplibium hirsutum*, and *M. aquatica*). There were a number of other species which occurred in four or more surveys however the percentage cover of these species was not high enough to meet the criteria (Fig. 17).

Crassula helmsii remained the most dominant species throughout the experiment in both grazed and ungrazed plots, with the next most abundant species having significantly lower proportional abundance. However, the dominance of *C. helmsii* was less distinct in the ungrazed plots, with lower mean difference and *t* values (Table 6; Fig. 18).

Table 6. The results of linear mixed effects models, to compare the proportional abundance of the most dominant species *Crassula helmsii* to the proportional abundance of the next most abundant species *Mentha aquatica* and *Epilobium hirsutum*. Data from grazed and ungrazed plot treatments were analysed separately.

Comparison		Treatment	Mean difference	SE	*t*	df	p
C. helmsii	*M. aquatica*	Grazed	-34.054	2.150	-15.838	47	< 0.001
C. helmsii	*M. aquatica*	Ungrazed	-13.424	1.554	-8.637	47	< 0.001
C. helmsii	*E. hirsutum*	Ungrazed	-16.687	2.115	-7.889	47	< 0.001

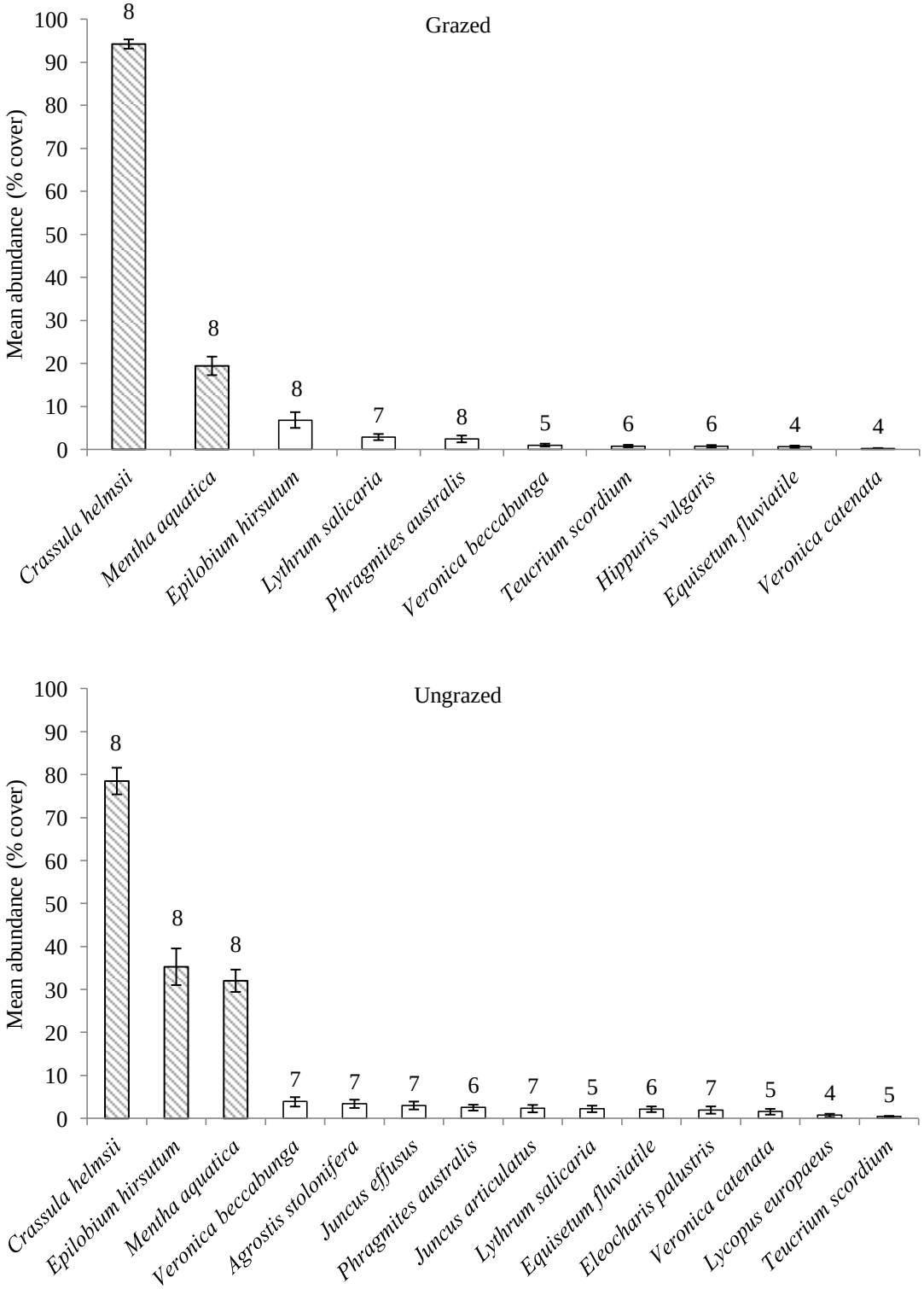


Figure 17. Abundant and frequently occurring plant species, recorded in grazed and ungrazed plot treatments. Species classed as 'abundant' (shaded bars) had a cover of 10% or higher in at least three of the six plots, in at least four of the eight sequential surveys. Species which were classed as 'frequent' (no fill) were recorded in at least four of the eight sequential surveys. Species are presented in rank order of mean abundance (± 1 SE), and data labels refer to the number of surveys in which each species was recorded.

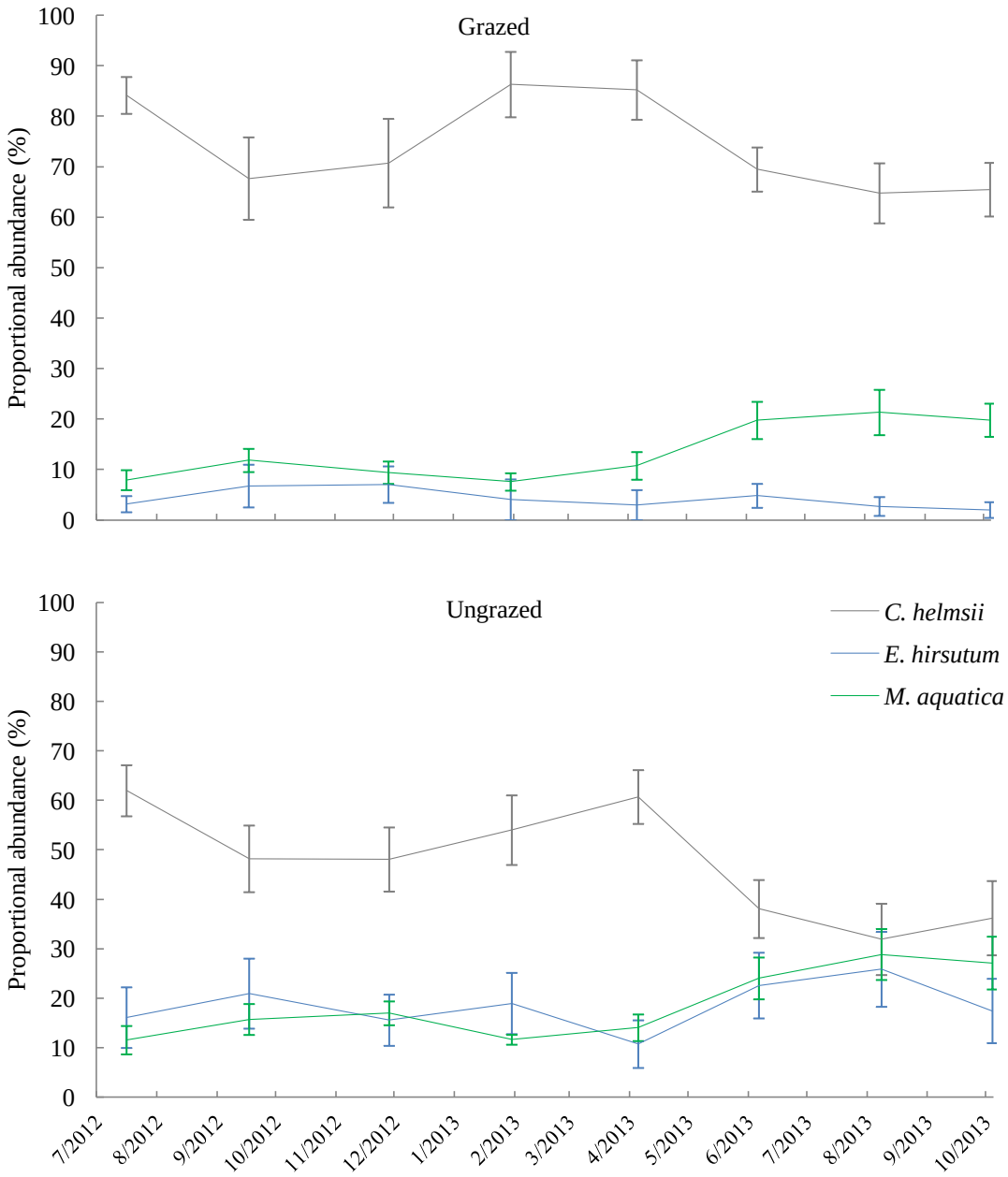


Figure 18. The mean proportional abundance (± 1 SE) of *Crassula helmsii*, *Epilobium hirsutum*, and *Mentha aquatica*, occurring together in (top) 'grazed' unfenced plots, and (bottom) 'ungrazed' fenced exclosures. Both graphs show the change in mean proportional abundance of these species, over the course of eight repeated surveys that were carried out between July 2012 and October 2013.

5.4. Discussion

The results reported in this chapter indicate that at this site, disturbance from grazing vertebrates had a significant impact on the structure of the plant community. The abundance of *C. helmsii* declined within the ungrazed plots, whilst maintaining very high abundance in the grazed plots. At the same time the abundance and diversity of the co-occurring plant community was higher in the ungrazed plots than in the grazed plots.

These results indicate that the presence of grazing vertebrates was advantageous for *C. helmsii* at this site. A likely explanation for these results is that *C. helmsii* benefitted from reduced interspecific competition in the grazed plots, because species which would have otherwise competed with *C. helmsii* were suppressed. Interestingly co-occurring species abundance was lower in the grazed plots right from the first survey in mid-July 2012, suggesting that competitors may have been grazed out earlier in the year by either sheep or wildfowl. Importantly maximum vegetation height was consistently higher in the ungrazed plots. It is hypothesised that the greater abundance of tall co-occurring plants in the ungrazed plots restricted access to light for *C. helmsii*, and resulted in its partial decline. *Crassula helmsii* also grew taller in the ungrazed plots and here it is interpreted that this was due to light limitation.

This explanation is consistent with Olff & Ritchie (1998) who discuss that in sites where nutrients and water are non-limiting, as was the case at Kingfishers Bridge (Beecroft *et al*. 2007; chapter three), tall plants which compete strongly for light are likely to dominate the plant community in the absence of grazing. At Kingfishers Bridge, *Epilobium hirsutum* had considerably higher abundance in the ungrazed plots compared to the grazed plots, and produced taller plants which were the main reason for the increased height of vegetation recorded in the ungrazed plots. Olff & Ritchie (1998) discuss that when grazing occurs at such sites, this creates a more open habitat which favours only those plants that are tolerant to grazing disturbance, and may not serve to increase species diversity. Other than *C. helmsii*, *Teucrium scordium* was the only plant species which had a higher mean abundance and frequency in the grazed plots. It is known that *T. scordium* prefers open habitat such as drawdown zones, and the unmanaged growth of taller, shading vegetation has been linked to the decline of *T. scordium* (Beecroft *et al.* 2007). This species is nationally rare, and classified as 'endangered' according to the IUCN Red List definition (Cheffings & Farrell, 2005; JNCC, 2010), and so its response in this experiment is of particular interest.

As well as reductions in competition, *C. helmsii* may have benefitted directly from grazing disturbance. For example trampling could have broken up the *C. helmsii* vegetation, creating more fragments which could have subsequently rooted. Additionally, the ability of *C. helmsii* to grow from nodes along creeping stolons (Dawson & Warman, 1987; EPPO, 2007), may have

compensated for shoot tip damage, and given this species a competitive advantage under grazed conditions (Milchunas *et al*. 1988; Díaz *et al*. 2007). However it is not known how extensively *C. helmsii* was grazed; although sheep and waterfowl have previously been seen to graze on the *C. helmsii* at this site (Cadbury, J. personal communication) during the experiment the *C. helmsii* did not appear to be 'cropped', instead it formed a dense vegetative mat of between 20 and 40 cm in height.

In laboratory experiments Langdon *et al*. (2004) showed that mats of *C. helmsii* vegetation can significantly suppress the germination success of other plant species. In the present study it is possible therefore, that the abundance and diversity of co-occurring vegetation was maintained at a lower level in the grazed plots, because grazing had selectively removed competitors, and because *C. helmsii* was preventing subsequent re-recruitment by supressing germination. Baiser *et al.* (2008), describe a similar interaction in North American deciduous forests, where increased browsing on the shrub layer by deer, had suppressed native species and favoured dense dominating growth of the non-native invasive grass *Microstegium vimineum*. The authors describe how regeneration of the native shrub layer was prevented both by strong competition from *M. vimineum* limiting seedling growth, and by continued over-browsing by deer.

An interesting further observation from this study site was that in February 2012 when the experiment began, the *C. helmsii* vegetation was considerably degraded, although no control had been attempted since September 2008 (Cadbury, J. personal communication). In January 2013 however the *C. helmsii* vegetation was thick and vigorous. Whilst data were not collected on this matter, it was noted that the water levels were considerably lower in February 2012 and the drawdown zone was exposed, whilst in January 2013 the drawdown zone was inundated. *Crassula helmsii* is classed as a hydrophyte (Hill *et al*. 2004), and so it is hypothesised here that the higher water levels during the second winter allowed *C. helmsii* to persist without degradation.

Additional information is required for further interpretation of this study. Firstly, additional information about the behaviour of the grazing animals and the condition of the experimental plots early in their establishment, would serve to confirm what animals grazed-out the competitors of *C. helmsii*. To gain this additional information the experiment would have to be run again, with frequent surveying during the early establishment phase, along with observations of what animals fed on the drawdown zone at this stage. Secondly, more empirical work is needed to determine whether *C. helmsii* directly benefits from grazing disturbance. This could be done using observational studies to determine which animals graze *C. helmsii* and how frequently, and by controlled clipping experiments (Kimball & Schiffman, 2003; Kohyani *et al.* 2009) to investigate how *C. helmsii* responds to shoot tip damage. Thirdly, it would be valuable to ascertain whether *C. helmsii* has a suppressing effect on *Teucrium scordium.* The results from the current study suggest that grazing favours the abundance of *C. helmsii*, and also favours the

persistence of endangered *T. scordium*. However based on abundance levels, *C. helmsii* strongly dominates. A study of this interaction would provide targeted information about the threat *C. helmsii* poses to *T. scordium* in its preferred habitat.

Traditionally light grazing by large vertebrates has been used in conservation to encourage plant species diversity, by supressing highly competitive and dominating plant species (Natural England, 2005; McBride *et al.* 2011). However, this current study is one of a number which report that grazing is linked to increased abundance of non-native invasive plants (Kimball & Schiffman, 2003; Baiser *et al.* 2008; HilleRisLambers *et al.* 2010; Relva *et al.* 2010). These results also concur with publications which highlight the role of disturbance as a factor facilitating non-native plant invasion and abundance, by making more resources available to the invader (Davis *et al.* 2000; Catford *et al.* 2012). In light of these non-native plant invasions, a new approach to management may be required, which considers how plant community competitive dynamics may have altered post-invasion (Seastedt *et al.* 2008; Firn *et al*. 2010).

The results of this study indicate that *C. helmsii* thrives under light grazing as this disturbance inhibits taller competitors. However, discouraging grazing by vertebrates and allowing taller vegetation to develop may run against other management objectives; in the case of Kingfishers Bridge the conservation of *Teucrium scordium* (Beecroft *et al.* 2007*)*. A better result may be reached, not by removing the grazing disturbance, but by altering the conditions and disturbance regime at the site to disadvantage the invasive plant (Firn *et al*. 2010). Based on observations from Kingfishers Bridge the following management regime could be trialled at the site: keeping the drawdown zone exposed during winter to stress *C. helmsii*, preventing or restricting grazing on the drawdown zone during spring to allow competitor species to germinate and establish, and using grazing later in the season to limit vegetation height.

6. Is available bare ground an important pre-requisite to high *C. helmsii* abundance?

6.1. Introduction

Reductions in the biomass of plants in a native community have been cited as a mechanism by which a non-native plant species may successfully invade habitats. For example, the theory of fluctuating resources (Davis *et al.* 2000) describes how reductions in native plant cover could increase the likelihood of invasion, due to reduced competition for resources. Not mutually exclusive of this, the driver-passenger theory of community change (MacDougall & Turkington, 2005) posits that some non-native invasive plants are the 'passengers' of community change, and become highly abundant under circumstance where they are able to adequately tolerate changes to the habitat which cause declines in native species. Both of these theories suggest that the absence of competition from the native plant community presents an opportunity for a non-native plant to invade and become highly abundant. It also follows that high abundance could be maintained, if the growth of the non-native invasive species created subsequent conditions of limited resources which inhibited later developing native competitors (Hager, 2004; Didham *et al*. 2005).

Bare ground is a good example of a habitat with low competition and high resource availability. Furthermore, there is interspecific variation in how well plants can tolerate environments in which disturbances create bare ground patches (Gross & Werner, 1982; Grime, 2001). Bare ground patches can be created naturally (Townsend *et al*. 2008). However, they can also be created by activities which are designed to control invasive and dominating vegetation, namely the physical removal and degradation of vegetation (Charudattan, 2001). Thus it is possible that the activities designed to control non-native invasive plants, may actually promote the abundance and persistence of some species (Hobbs & Huenneke, 1992). In line with this, studies which have monitored community regeneration following the clearance of invasive vegetation, have reported subsequent colonisation by secondary invaders and considerable re-colonisation by the original non-native invasive species (Hulme & Bremner, 2006; Mason & French, 2007; Jäger & Kowarik, 2010; Kettenring & Adams, 2011).

To take advantage of available bare ground, it follows that a non-native invasive species must be able to move into the space before co-occurring native species. Traits such as fast growth, extensive aboveground production, and lateral spread may aid in competition (Grime, 2001), and in the literature fast growth rate and high vegetation production are traits which have been associated with non-native invasive species (Hussner, 2009; Dawson *et al*. 2011; Graebner *et al*.

2012; Skálová *et al*. 2013). An advantage could also be gained if a non-native invasive species begins growth earlier in the season than other species, or responds faster to favourable growing conditions (Wainwright *et al.* 2012; Wainwright & Cleland, 2013). This could lead to greater competitive dominance later in the season, if earlier expansion allowed the non-native invasive species to achieve a larger size than other species (Abraham *et al*. 2009; Dickson *et al.* 2012). Furthermore, non-native invasive plants with a seasonal growth period which extends later than its competitors, could gain an advantage by expanding into available space whilst other species had stopped (Wolkovich & Cleland, 2011).

Crassula helmsii has several traits which may be advantageous when competing for available bare ground, including high relative growth rate (Minchin, 2008; Hussner, 2009), high vegetative productivity, and a horizontal growth habit (Dawson & Warman, 1987; EPPO, 2007), allowing mature plants to move into adjacent space on creeping stolons. Being a perennial which is winter green and experiences little winter die-back (Preston & Croft, 1997; EPPO, 2007; Minchin, 2008), *C. helmsii* may also have a longer growing season than many annuals or deciduous perennials. Furthermore, schemes to remove invasive *C. helmsii* vegetation report a high incidence of re-colonisation (Bridge, 2005; Gomes, 2005; Wilton-Jones, 2005), suggesting that the conditions created during removal are highly suitable for subsequent *C. helmsii* growth.

Two separate experiments are described in this chapter, which both assess how well *C. helmsii* can compete for available bare ground, compared to native plant species which inhabit the same habitats. Firstly, a common garden experiment is described which investigated the rate at which *C. helmsii* could expand horizontally into unoccupied bare ground, when grown in paired combinations with native species, and when grown singularly in a control. Secondly, a field experiment is described which monitored the abundance of *C. helmsii*, as it naturally colonised and grew within sample plots in the field. This experiment tested whether plots which initially had bare ground (lower competition), were colonised more quickly by *C. helmsii* and developed higher *C. helmsii* abundance, than plots which had initially been planted with native vegetation (higher competition). Within these two experiments, the following questions were asked:

Common garden experiment: 1) Is there a difference in expansion rate between *C. helmsii* and the native species? 2) Is the expansion rate of *C. helmsii* affected by interspecific competition from native plants? 3) Are the expansion rates of the native plant species affected by competition from *C. helmsii*? 4) Which paired species becomes more abundant, *C. helmsii* or the native plant?

Field experiment: 1) Does plot treatment have an effect on the speed of *C. helmsii* colonisation? 2) Does plot treatment have an effect on the abundance of *C. helmsii*?

Assessing how well *C. helmsii* can compete for bare ground relative to native species has important relevance for the management of habitats which have been invaded by this non-native plant. This is because if bare ground favours *C. helmsii* abundance over other species, then management activities which create bare ground could inadvertently encourage higher *C. helmsii* abundance.

6.2. Methods and materials: Common garden experiment

6.2.1. Experimental design

Crassula helmsii was grown in paired treatments with three different native species *Anagallis tenella*, *Hypericum elodes*, and *Hydrocotyle vulgaris*. These three native species were chosen because they had been found at the site of the field-based experiment reported in this chapter (sections 6.4. and 6.5.), and thus were known to naturally co-occur with *C. helmsii*. Furthermore, *A. tenella*, *H.elodes*, and *H. vulgaris* were specifically chosen out of the pool of species at Little Sea because they possessed growth habits similar to *C. helmsii*; that of low growing, creeping, perennial herbs of wetland margins (Hill *et al*. 2004; Stace, 2010). Therefore, the competitive strength of *C. helmsii* was being tested, relative to native species which occupied a similar niche and could potentially be displaced by *C. helmsii* in the field.

Ahead of the set-up of the common garden experiment, in May 2012 stock cultures were planted of each of the chosen test species. These stock cultures were planted within plastic containers (35 cm width x 50 cm length x 20 cm height), two containers per species, which were filled with aquatic plant compost and saturated with tap water to mimic wetland habitat substrate. *A. tenella*, *H. vulgaris*, and *H. elodes* plants were purchased from a commercial aquatic plant specialist (www.lilieswatergardens.co.uk) in May 2012, and additional plants were purchased from the same supplier in July 2012 to expand the cultures. *Crassula helmsii* for this experiment had originally been collected in March 2011 from Studland Nature Reserve in Dorset (OS grid ref: SZ 035 835). The stock cultures were kept in an outdoor location at Bournemouth University, Dorset (OS grid ref: SZ 073 937). To shield the cultures from seed rain and detritus, translucent lids were placed over the plastic containers, which were propped open to allow for transpiration and to prevent overheating.

The common garden experiment ran for one year, beginning on 14th September 2012 and ending on 14th September 2013. Between-species differences in the patterns of seasonal growth activity can influence the outcome of competitive interactions (Wolkovich & Cleland, 2011; Wainwright *et al.* 2012). Thus the timing of this experiment, at the end of summer, meant that how long each species could continue growing into autumn and how quickly they could resume growth the following spring, was predicted to have an effect on their overall ability to compete for available bare ground.

Crassula helmsii was planted in pair-wise combinations with each of the chosen native species, and all four species were planted in monocultures as a control. Thus there were seven different planting treatments in this experiment. Each planting treatment was replicated five times, producing a total of 35 individual replicates. Each replicate was contained within an individual planting basket, designed for aquatic pond plants, which were 12 cm in height and 16 cm in

diameter. Each basket was lined with thin porous fabric and filled to 5 mm below the brim with aquatic plant compost, which had been homogenised by hand. The same amount was planted into each replicate by cutting circular plugs of the cultured vegetation 55 mm in diameter, with trailing stolons trimmed back to a maximum of 5 cm. These plugs of vegetation were cut from the stock cultures and planted two per basket. A pair-wise treatment therefore contained one plug of a native species and one plug of *C. helmsii*, whereas a control contained two plugs of one species. Plugs were planted in the middle of the basket directly next to each other, and at the start of the experiment there was available bare compost surrounding the plugs for plants to grow into (Fig. 19).

Figure 19. Photograph showing the composition of an individual replicate in this experiment. This particular replicate shows the experimental treatment of *Crassula helmsii* and *Hydrocotyle vulgaris* planted in a pair-wise combination.

The replicates were contained within a single fibreglass pond, in order that the depth and nutrient status of the water was homogeneous for all replicates. The pond was filled with tap water to 10 cm depth; this was deep enough to saturate the compost in the pots thus mimicking marginal wetland conditions, but it was not so deep as to submerge the plants, which would have compromised the independence of the replicates by allowing vegetation fragments to float between the planting baskets. During the course of the experiment an outflow was kept open on

the side of the pond which evacuated water if the depth increased above 10 cm, and more tap water was added to the pond if the depth decreased below 10 cm.

The fibreglass pond was located in an outdoor compound, at Bournemouth University. There was no supplementary temperature or light control, and thus the plants experienced temperatures (Fig. 20) and a photoperiod which varied according to natural daily and seasonal variation. The outdoor compound was located adjacent to a building, facing north-east. Shading from the adjacent building caused a slight photogradient across the experimental area, and so replicates were placed within the pond in a random arrangement, and were re-arranged every four weeks. During the morning, this photogradient was estimated to be a difference in PAR of 1.6 x10µmol.m^{-2}.s^{-1}. In the afternoon this photogradient was less pronounced, estimated to be a difference in PAR of 0.5 x10µmol.m^{-2}.s^{-1}. The extent of this gradient was estimated by taking PAR measurements (using a Skye Instruments Ltd. PAR quantum sensor) twice daily at 10:00 and 16:00 hours, at locations nearest to and furthest from the building, over five weeks from 26th September to 15th October 2012, and 4th to 18th February 2013. The mean difference in PAR in the morning and in the afternoon was calculated from these data.

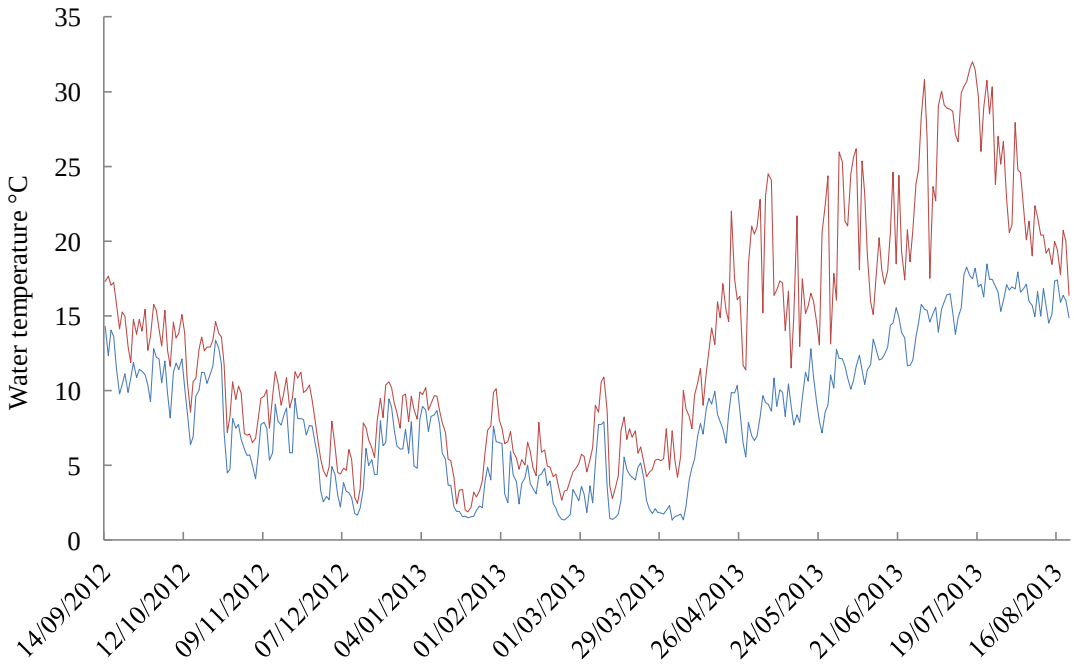


Figure 20. The daily maximum (red) and minimum (blue) temperature of water within the pond used for the planting experiment, during the year in which the experiment was run. Temperature was recorded using a Tingtag Plus 2 data logger (© Tinytag, 2013).

6.2.2. Data collection

To measure the expansion in horizontal growth, the cover of vegetation in each replicate as viewed from directly above, was recorded at the start of the experiment and then once every four weeks until the end of the experiment. This gave a total of 14 repeated measurements of cover for each replicate. Documenting the horizontal spread over time served as a non-destructive method of estimating plant growth.

To record the horizontal cover, photographs were taken of each replicate, and the horizontal area of cover was calculated from these. All photographs were taken using a Nikon COOLPIX P100 digital camera mounted on a tripod set to 50 cm high, directly above the planting basket. The fully automatic mode was used for all photos, and produced JPEG images at a resolution of 300 dpi. Horizontal cover was measured from the photographs, by digitally overlaying a 1000 x 1000 pixel grid onto each photograph, and counting the number of grid cells a plant occupied. A 1000 x 1000 grid cell size was chosen as it was judged to provide the best compromise between the resolution at which the cover was measured, and the time taken to count the grid cells. The computer program ImageJ (Schneider *et al.* 2012) was used for counting; grid cells which contained vegetation were marked as 'in' by clicking with the mouse, and the plugin 'cell counter' provided a tally of the number of grid cells based on the number of mouse clicks. A binary 'in/out' method was used, so that grid cells which contained any amount of vegetation were classed as 'in', and only grid cells which were completely empty of vegetation were classed as 'out'. For the paired species replicates, a separate tally was counted for the two different species in the pair. For the control treatments it became increasingly difficult to distinguish between the two individual plugs as they grew together, and so a single tally was counted of both plugs together and this figure was divided by two to give an average per individual.

As plants in the paired-species treatments expanded, competition for space led to species overlapping in their cover. When this occurred, one species could be obscured in photographs due to overtopping by the competing species. To account for this, when photographs were being taken the amount of overtopped vegetation was estimated and recorded as a percentage, relative to the amount that was visible from above. The number of grid cells that this overtopped vegetation represented, was subsequently calculated from the number of grid cells that the photograph-visible vegetation was counted to represent. These two counts were added together to give a total grid cell count for each species in each replicate. A limitation to this method was that it was not possible to determine the extent of overtopping between the two plugs of the same species in the control treatments.

At the end of the experiment, the maximum above-ground height of each species in each replicate was measured in millimetres, with a precision of one millimetre. The above-ground biomass was then harvested by cutting the stems at soil level. Vegetation was dried at 90˚C for 48 hours, and then each species in each replicate was separately weighed to a precision of ± 0.001 g to obtain final dry weight values.

6.2.3. Statistical analysis

The raw horizontal cover data, measured as the number of grid cells, were used to calculate the proportion of space a species occupied, relative to the total amount of space it could occupy. For the paired-species treatments this was done using the formula:

Proportional cover = (cover of species / total cover)*100

Whereas for the monoculture control treatments this was done using the formula:

Proportional cover = ((cover of species / 2) / total cover)*100

In both formulae, 'total cover' represents the summed cover of all plant grid cells, occupied and unoccupied. Using proportional data accounted for the fact that the extent of cover of each species was ultimately limited by the area of the plant pot.

These data were used to calculate a rate for the increase in proportional cover of each species, in each planting treatment. To do this, expansion rate was equated to the value of the slope of the fitted line in a linear regression model, with proportional cover as the dependent variable and time in days as the independent variable. Separate linear regression models of proportional cover predicted by time were calculated for each replicate, providing a slope value for the expansion rate for each species in each individual replicate.

In this experiment *C. helmsii* showed two distinct growth phases during the year, one beginning in autumn 2012 and one beginning in summer 2013, with a decline in vegetative cover between these growth phases. This meant that the relationship between *C. helmsii* horizontal cover and time, over the full year of the experiment, was non-linear. For this reason it was decided to calculate separate slope values for the two growth phases. In the first growth phase slope values were calculated for expansion occurring between 14th September 2012 and 4th January 2013, and in the second growth phase slope values were calculated for expansion occurring between 24th June 2013 and 14th September 2013. In this way, the expansion rates were only calculated for the parts of the year when *C. helmsii* was actually expanding, not when it was showing vegetative decline. For each of the native species, the same growth phases were adhered to because these represented the times during the year when *C. helmsii* was expanding, thus competing more strongly with the native species.

Is there a difference in expansion rate between C. helmsii and the native species?

One-way ANOVA was used to test whether there was a significant difference in the mean slope values between the test species, when each species was grown singularly in the control. Thus whether there was a difference in expansion rate between the species, in the absence of interspecific competition. Tukey's hsd post-hoc tests were used to make specific comparisons between the mean slope value of *C. helmsii* and each of the native species. The tests were conducted separately for the slope values calculated in the first and second growth phases.

Is the expansion rate of C. helmsii affected by interspecific competition from native plants?

One-way ANOVA was used to test whether there was a significant difference in the mean slope values of *C. helmsii* grown in different treatments, indicating a between-treatment difference in the expansion rate. Tukey's hsd post-hoc tests were used to make specific comparisons between the mean slope value of *C. helmsii* in the control and the mean slope value in each of the paired species treatment, translating to a difference in the expansion rate between *C. helmsii* grown in on its own and *C. helmsii* grown with interspecific competition. These tests were conducted separately for the slope values calculated in the first and second growth phases.

Are the expansion rates of the native plant species affected by competition from C. helmsii?

Data from each native species was analysed separately. Independent *t*-tests were used to test whether there was a significant difference in the mean slope value between the native species in the control and the native species when paired with *C. helmsii*. This identified whether there was a significant difference in the expansion rate between the native species when grown in on its own, versus when competing with *C. helmsii*. The tests were conducted separately for the slope values calculated in the first and second growth phases.

Which paired species becomes more abundant, C. helmsii or the native plant?

Tests were conducted to determine whether there was an asymmetry in the abundance of *C. helmsii* compared to the native species it was paired with; a large asymmetry indicating that the more abundant species was out-competing the other. Two different measures of abundance were tested, horizontal cover and dry weight.

Horizontal cover was tested using the proportional cover data for the end of the first growth phase (4th January 2013), and for the end of the second growth phase (14th September 2013) which was also the end of the experiment. In both cases paired *t*-tests were conducted to test whether there was a significant difference in horizontal cover between *C. helmsii* and the native species it was paired with.

Dry weight data was only available for the end of the experiment (14th September 2013), because only at this point was destructive sampling conducted. The dry weight data did not meet the assumption that the numeric difference between the paired values was normally distributed (Field *et al*. 2012), and so Wilcoxon's signed-rank tests were used to test whether there was a significant difference in dry weight between *C. helmsii* and the native species it was paired with.

6.3. Results: Common garden experiment

Is there a difference in expansion rate between C. helmsii and the native species?

In the weeks following planting, *C. helmsii* rapidly spread out to occupy available bare soil. This was in contrast to the native species, where *A. tenella* showed a slighter increase in cover, and *H. elodes* and *H. vulgaris* showed declines in cover (Fig. 21). Indeed, in the first growth phase there was a strong significant difference in the mean slope value between the test species in the control treatments ($F_{(3, 16)} = 63.815$, $p < 0.001$). Tukey's hsd post hoc tests showed that the mean slope value was significantly higher for *C. helmsii* than for *A. tenella* (mean diff. = 0.141, p = 0.001), *H. elodes* (mean diff. = 0.301, $p < 0.001$), and *H. vulgaris* (mean diff. = 0.385, $p < 0.001$). Both *H. elodes* and *H. vulgaris* had negative slope values for this phase indicating negative expansion and thus vegetative decline.

The three native species showed the most pronounced increases in cover during spring and summer 2013, and at this time the increase in *C. helmsii* cover was slighter (Fig. 21). Reflective of this, in the second growth phase there was a significant difference in the mean slope value between the test species ($F_{(3, 16)} = 28.701$, $p < 0.001$), with Tukey's hsd post hoc tests showing that the mean slope value was significantly lower for *C. helmsii* than for and *H. elodes* (mean diff. = -0.303, $p < 0.001$), and that there was no significant difference between *C. helmsii* and *A. tenella* (mean diff. = -0.066, p = 0.401), or *H. vulgaris* (mean diff. = 0.066, p = 0.682).

Is the expansion rate of C. helmsii affected by interspecific competition from native plants?

In the first growth phase, the *C. helmsii* control increased in cover rapidly for the first month and then was shown to plateau at approximately 50 % proportional cover, whilst in the paired-species treatments *C. helmsii* was found to keep increasing throughout the measured time period (Fig. 21). In this growth phase there was a significant difference in the mean slope value between the four planting treatments ($F_{(3, 16)} = 27.540$, $p > 0.001$), in which the slope in the control treatment was significantly lower than the slope when *C. helmsii* was paired with *A. tenella* (mean diff. = -0.202, p = 0.001), *H. elodes* (mean diff. = -0.198, $p < 0.001$), and *H. vulgaris* (mean diff. = -0.320, $p < 0.001$).

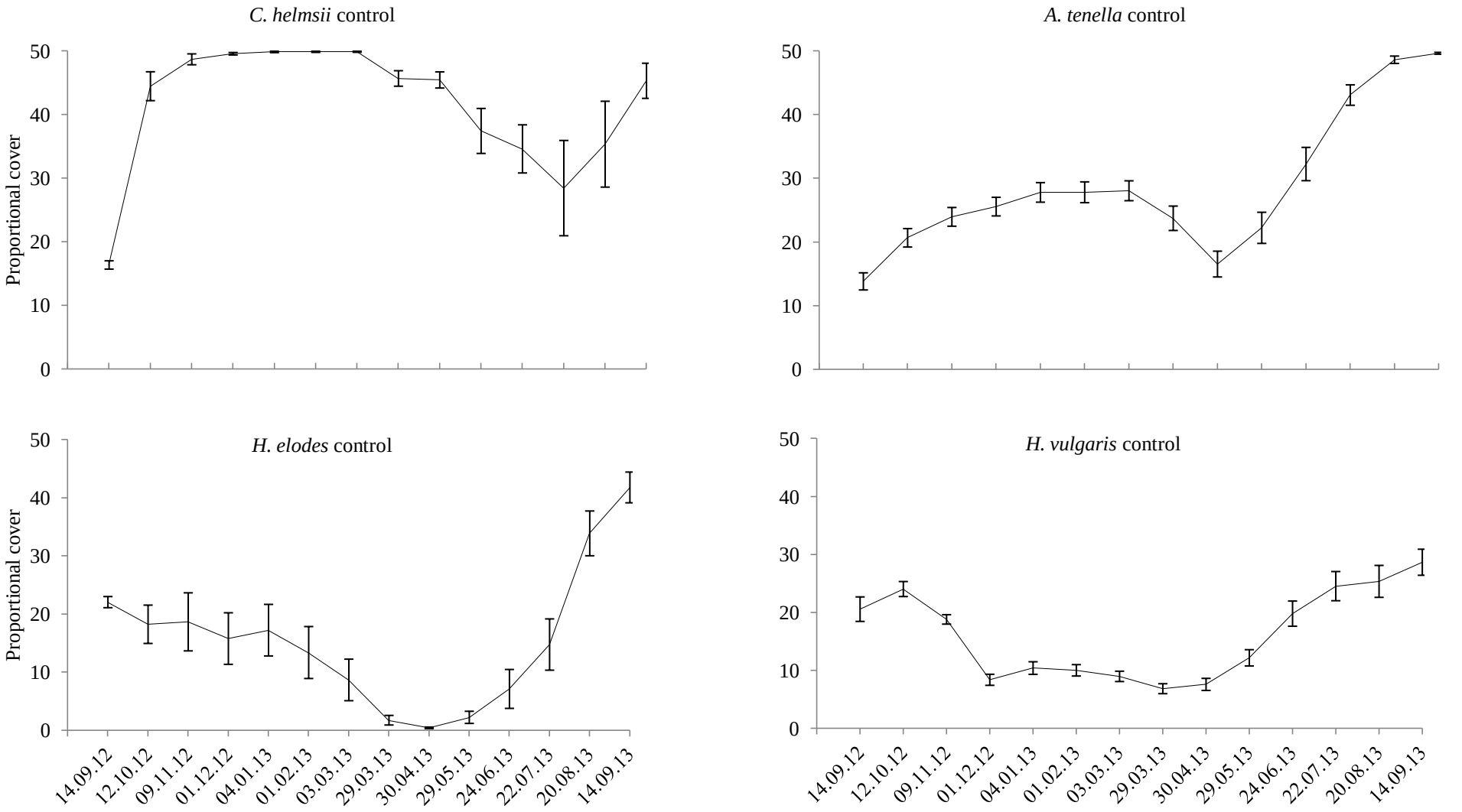
In the second growth phase expansion was slower in all treatments, compared to the first growth phase (Fig. 21). There was no significant difference in the mean slope value between the four planting treatments ($F_{(3, 16)} = 1.952$, p = 0.162). However, the mean slope for *C. helmsii* paired with *H. vulgaris* was lower than for the other three treatments: control (mean diff. = -0.252, p = 0.241), *A. tenella* (mean diff. = -0.283, p = 0.164), *H. elodes* (mean diff. = -0.173, p = 0.545).

Are the expansion rates of the native plant species affected by competition from C. helmsii?

Anagallis tenella. The mean slope value was significantly higher in the control than in the paired treatment, both in the first growth phase ($t_{(8)}$ = 8.788, p < 0.001), and in the second growth phase ($t_{(8)}$ = 2.860, p = 0.021).

Hypericum elodes. There was no significant difference in the mean slope value between the control and the paired treatment in either the first growth phase ($t_{(8)}$ = 0.547, p = 0.599), or in the second growth phase ($t_{(8)}$ = -0.392, p = 0.706).

Hydrocotyle vulgaris. In the first growth phase, there was no significant difference in the mean slope value between the control and the paired treatment ($t_{(4.971)}$ = 0.587, p = 0.583). In the second growth phase, the mean slope value was significantly higher in the paired treatment than in the control ($t_{(8)}$ = -4.292, p = 0.003).



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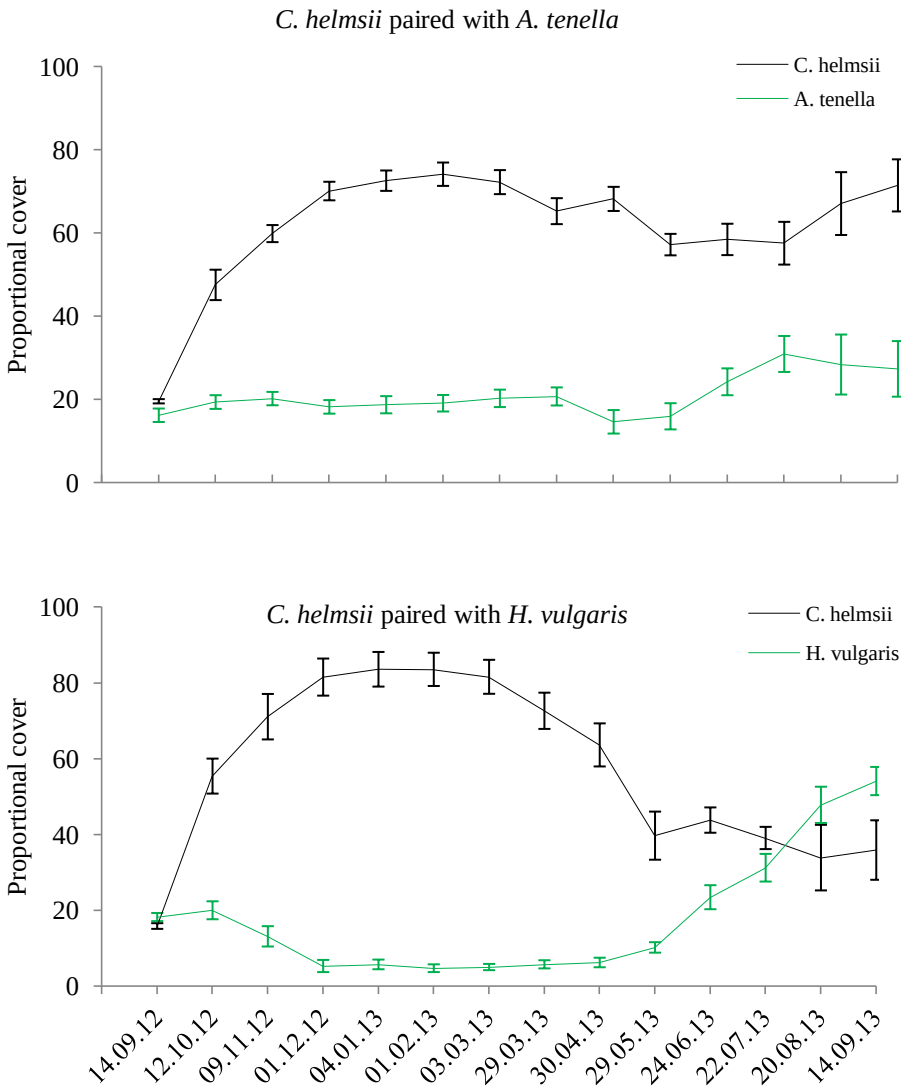


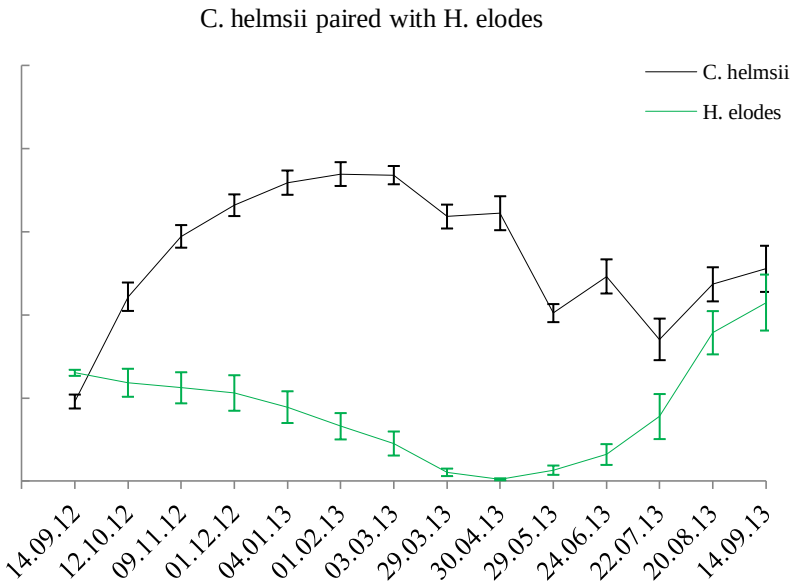


Figure 21 (and previous page). The mean horizontal cover (± 1 SE) over time of four species: *Crassula helmsii*, *Anagallis tenella*, *Hypericum elodes*, and *Hydrocotyle vulgaris*. For each species, the growth over time is shown for a single plug of vegetation which was either paired with itself in a monoculture (control), or paired in a two-species combination.

Which paired species becomes more abundant, C. helmsii or the native plant?

At the end of the first growth phase the mean cover was significantly higher for *C. helmsii* than *A. tenella* ($t_{(4)}$ = 13.078, p < 0.001), significantly higher for *C. helmsii* than *H. elodes* ($t_{(4)}$ = 9.033, p = 0.001), and significantly higher for *C. helmsii* than *H. vulgaris* ($t_{(4)}$ = 13.598, p < 0.001), when these species were grown in paired treatments (Fig. 22). At the end of the second growth phase, the mean cover was significantly higher for *C. helmsii* than for *A. tenella*, when these species were grown in a paired treatments ($t_{(4)}$ = 3.410, p = 0.027). There was no significant difference in mean cover between *C. helmsii* and *H. elodes* ($t_{(4)}$ = 0.667, p = 0.541), or between *C. helmsii* and *H. vulgaris* ($t_{(4)}$ = -1.660, p = 0.172) (Fig. 22).

At the end of the experiment, the median dry weight was consistently higher for *C. helmsii* than for the paired native species (Table 7), however this between-species difference was not statistically significant; *C. helmsii* with *A. tenella* (p = 0.080), *C. helmsii* with *H. elodes* (p = 0.080), *C. helmsii* with *H. vulgaris* (p = 0.345), as there was considerable between-replicate variation (Table 7). Based on the median maximum height data for the end of the experiment, *C. helmsii* grew taller than *A. tenella* but *H. elodes* and *H. vulgaris* grew taller than *C. helmsii* (Table 8).

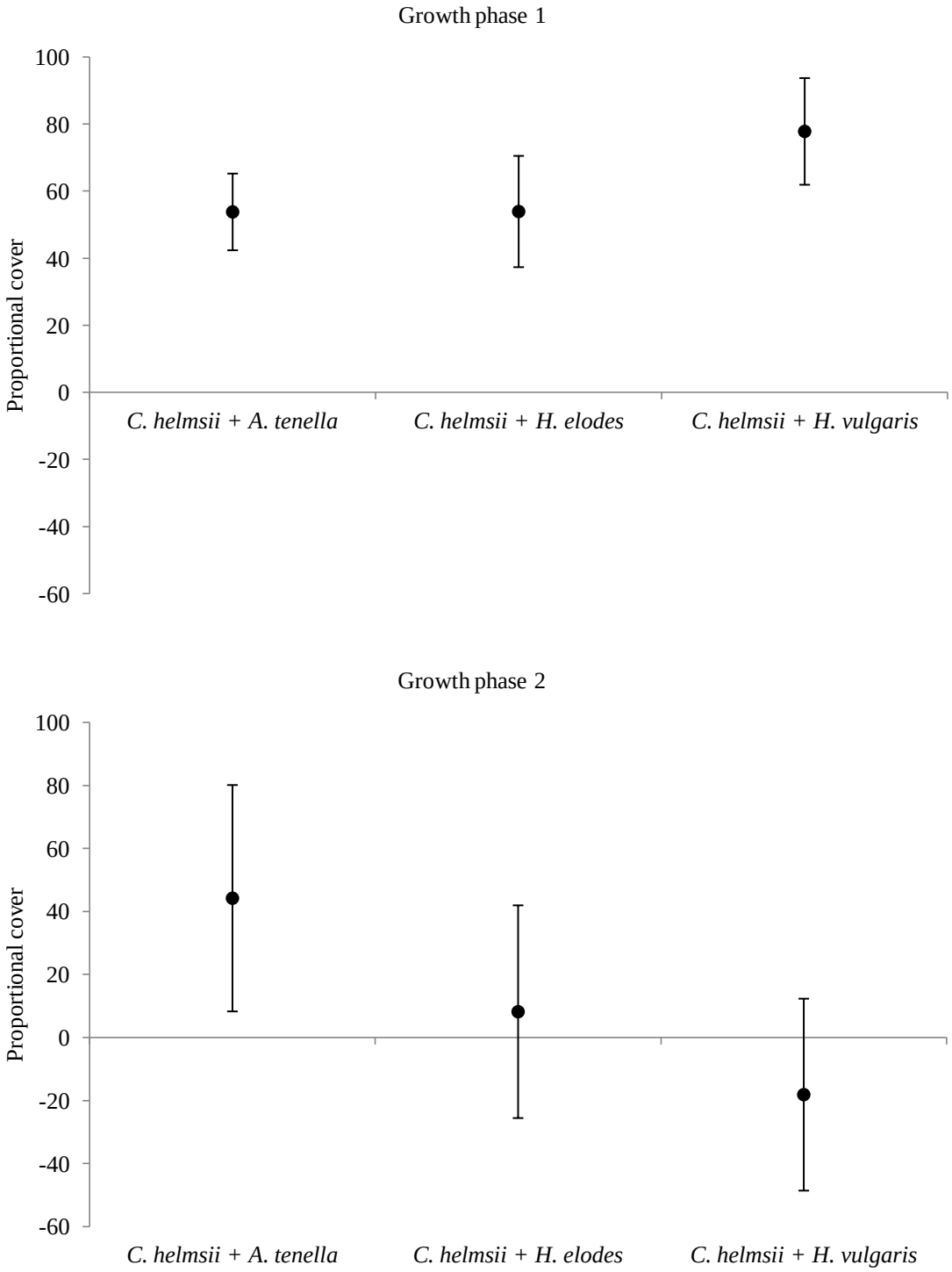


Figure 22. These graphs show the outcomes of paired *t*-tests that were used to test for a significant difference in mean cover between *Crassula helmsii* and the species it was paired with. Three separate paired species treatments are shown; (left) *C. helmsii* with *Anagallis tenella*, (middle) *C. helmsii* with *Hypericum elodes*, and (right) *C. helmsii* with *Hydrocotyle vulgaris.* In these graphs, where the confidence interval of the mean remains above 0 'proportional cover' on the Y axis this is indicative of a result where *C. helmsii* had a significantly higher cover than the species it was paired with. Where the confidence interval of the mean falls across the X axis (above and below 0 cover), this is indicative of a result where there was no significant difference in cover between the paired species.

Table 7. The dry weight of vegetation harvested at the end of the experiment. The data are shown for three separate paired treatments; *Crassula helmsii* paired with *Anagallis tenella*, *C. helmsii* paired with *Hypericum elodes*, and *C. helmsii* paired with *Hydrocotyle vulgaris.*

Planting treatment	Species	Dry weight (g)		
		Median	Min	Max
C. helmsii + A. tenella	*C. helmsii*	4.428	2.651	6.331
	A. tenella	1.706	1.029	2.909
C. helmsii + H. elodes	*C. helmsii*	2.297	2.090	2.575
	H. elodes	0.859	0.445	2.421
C. helmsii + H. vulgaris	*C. helmsii*	2.607	0.206	3.598
	H. vulgaris	1.287	0.624	2.340

Table 8. The maximum height of species as measured at the end of the experiment. The data are shown for three separate paired treatments; *Crassula helmsii* paired with *Anagallis tenella*, *C. helmsii* paired with *Hypericum elodes*, and *C. helmsii* paired with *Hydrocotyle vulgaris.*

Treatment	Species	Max height (mm)		
		Median	Min	Max
C. helmsii + A. tenella	*C. helmsii*	50	45	53
	A. tenella	33	31	41
C. helmsii + H. elodes	*C. helmsii*	42	36	55
	H. elodes	56	37	91
C. helmsii + H. vulgaris	*C. helmsii*	47	11	55
	H. vulgaris	54	36	59

6.4. Methods and materials: Field experiment

6.4.1. Experimental design

Experimental plots were set up along a stretch of the drawdown zone bordering Little Sea lake, within Studland Nature Reserve, Dorset, UK (OS grid ref: SZ 035 835). Studland nature reserve is owned and managed by the National Trust, and encompasses successional sand dune habitat transitioning into wet heathland. Little Sea is a land-locked lake which exists within this transitional habitat, receiving fresh, acidic oligotrophic water as run-off from the heathland (JNCC, n.d).

This site was chosen for the experiment due to its recent history of *C. helmsii* colonisation and subsequent control efforts. *Crassula helmsii* had been found on the drawdown zone of Little Sea in August 2007, and was described as being frequent but not dominant. In October 2008 *C. helmsii* was cleared by hand from three adjacent patches of this drawdown zone, and these patches were covered with black plastic sheeting to block access to light and thus prevent re-growth (Peters, A. personal communication). In May 2011 it was judged that all *C. helmsii* under the plastic would have been destroyed and so the plastic sheeting was removed. All vegetation underneath the plastic had degraded revealing bare mud, but despite this success, *C. helmsii* was still found growing around the edges of the plastic and elsewhere in the lake, meaning that there was a high potential for this species to re-colonise the cleared area.

The experiment was set up on the 28th of June 2011 following removal of the plastic sheeting. As described above, three patches of bare mud had been created which were 24, 3, and 16 m^2 in area. These patches all occurred within an 80 m stretch of the drawdown zone and were at an equal distance from the edge of the water. These three patches were considered as a single site due to their close proximity to one another, because they experienced the same abiotic conditions, and were exposed to the same plant species pool. Within these bare mud patches, 75 square plots of 50 cm^2 were marked out using string and bamboo canes (Fig. 23). As a way of trying to ensure that all plots received similar levels of water and nutrients, the plots were specifically placed on bare mud which was damp but not inundated and was free of any remaining rotting wood. For the majority of the experiment the plots were situated in marginal habitat above the level of the water, however visits to the site found that the plots were inundated in June 2012 and in March 2013.

The 75 plots were divided into three treatments groups, with 25 plots per treatment group. Individual plots were randomly assigned to one of the three treatment groups, ensuring that there was a random distribution of the three treatments across space. The three treatment groups were:

1) Left as bare ground: nothing was done to the plots during the set-up. At the start of the experiment they consisted of 50 cm^2 of bare mud.

2) Planted with grass dominated vegetation: at set-up a 25cm^2 square of turf was transplanted into the middle of the plots. This turf consisted mainly of the grass species *Agrostis stoloniefera*.

3) Planted with moss dominated vegetation: at set-up a 25cm^2 square of turf was transplanted into the middle of the plots. This turf consisted mainly of the moss species *Sphagnum cuspidatum.*

A. stolonifera and *S. cuspidatum* were selected because they were growing in abundance at the site immediately surrounding the cleared experimental area, and were therefore judged to be strong competitors in that plant community. The grass dominated turf contained at least 80 % *A. stolonifera*, and the moss dominated turf contained at least 90 % *S. cuspidatum*. Because the mature vegetation was obtained from a natural community it was not possible to collect mono-specific turf sections. Within the turf were also small amounts of *Hydrocotyle vulgaris*, *Hypericum elodes*, *Anagallis tenella* and *Juncus* spp., and some mixing of *S. cuspidatum* and *A. stolonifera*. Importantly all turf was checked to ensure that it did not contain any fragments of *C. helmsii*.

Figure 23. Photograph showing a section of the drawdown zone at Little Sea lake, shortly after the 75 experimental plots were set-up in summer 2011. Plots of all three treatments groups are included in the image; (1) bare ground, (2) grass dominated, and (3) moss dominated.

6.4.2. Data collection

The 75 experimental plots were surveyed during the spring, summer, and autumn of 2011 to 2013. Surveys were planned to be repeated once every six weeks during these seasons. However the logistics of reaching the experimental area, which could be accessed only by boat, meant that the length of time between surveys varied between four and seven weeks. In total the survey was repeated 13 times: 24th August and 9th November 2011, 24th April, 14th June, 24th July, 29th August, 2nd October, and 8th November 2012, 8th May, 14th June, 12th July, 13th August, and 26th September 2013.

For each survey, data were collected on the percentage cover of *C. helmsii*, the species identity and the percentage cover of co-occurring vegetation. The spatial location of each experimental plot was recorded with an accuracy of c. 10 cm, using a Leica Viva differential GPS rover and reference station.

6.4.3. Statistical analysis

Does plot treatment have an effect on the speed of C. helmsii colonisation?

A test was conducted to see if there was an effect of plot treatment, on how quickly *C. helmsii* colonised a plot. The speed of colonisation was quantified by determining for each of the 75 experimental plots, the survey in which *C. helmsii* was first recorded to occur. This dataset had a large positive skew which could not be overcome using data transformation methods, and so a Kruskal-Wallis test was used to analyse whether there was a significant difference between bare ground, grass dominated, and moss dominated plot treatments, regarding when *C. helmsii* was first recorded.

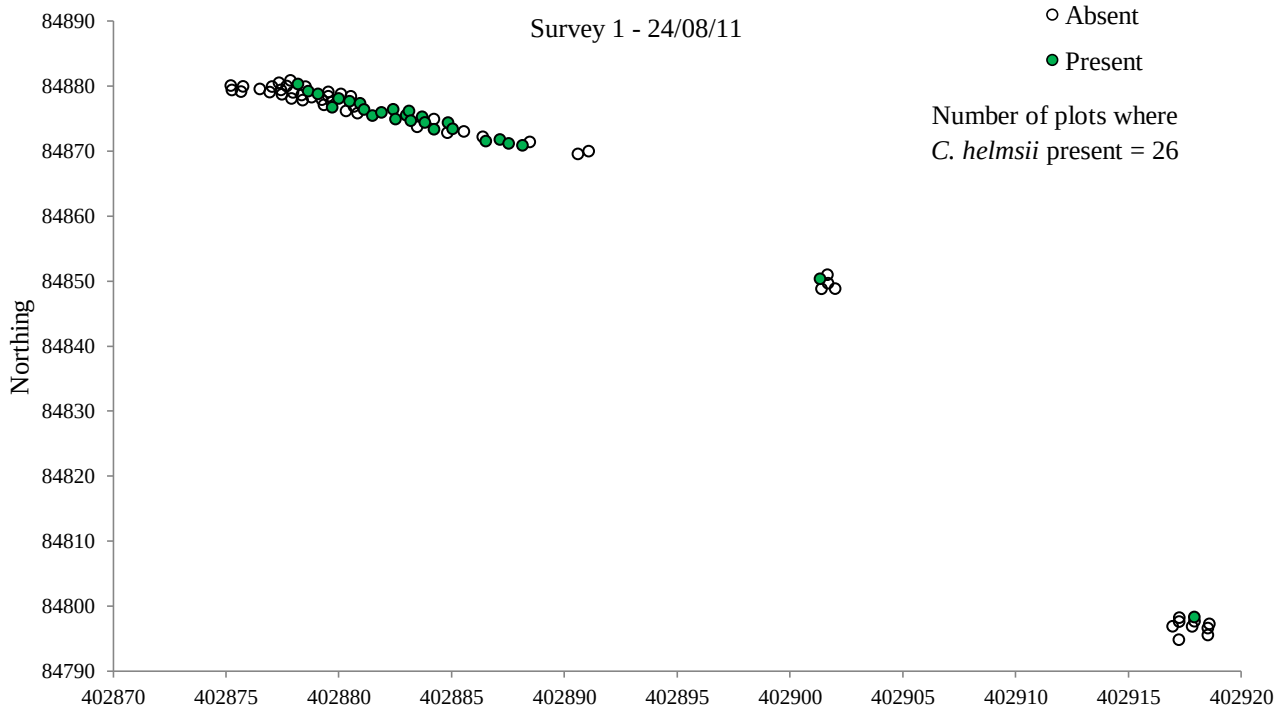
Does plot treatment have an effect on the abundance of C. helmsii?

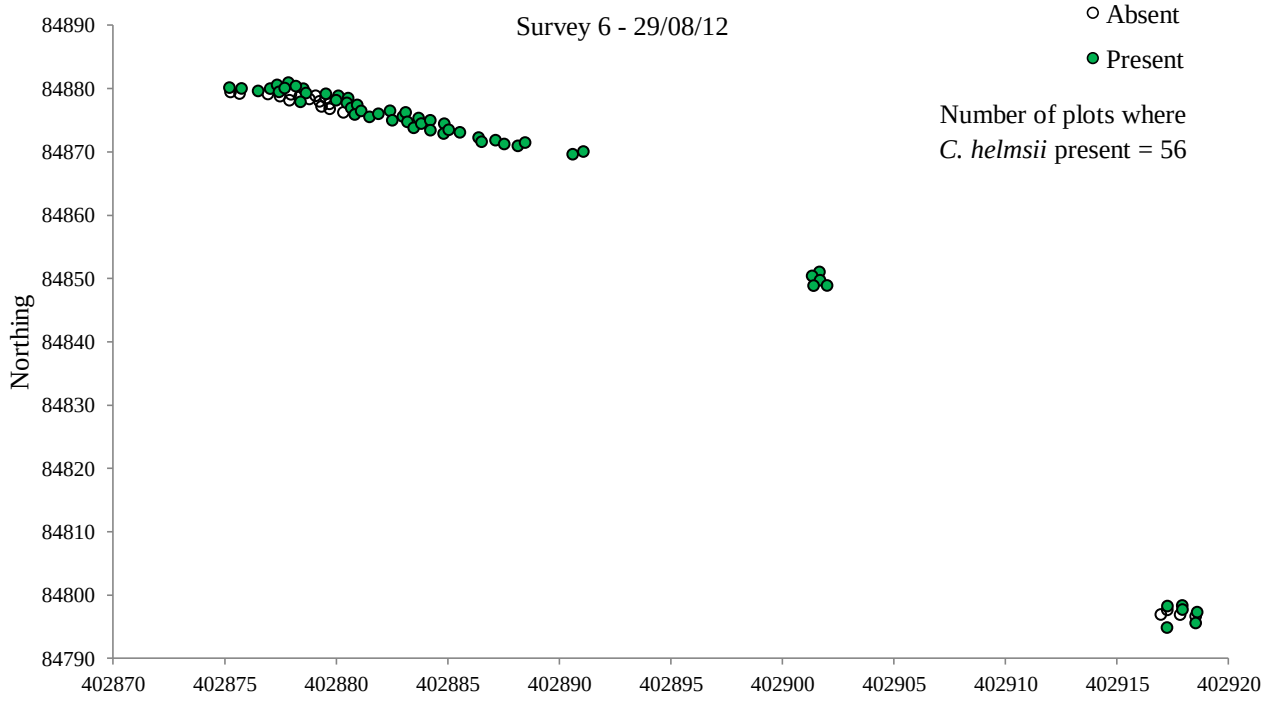
Data from all 13 surveys were included in a single linear mixed effects model, to test whether plot treatment had a significant effect on mean *C. helmsii* abundance. Before analysis, the percentage cover data for *C. helmsii* were arcsine transformed (see Chapter 2, section 2.2). The linear mixed effects model was run using the R package 'nlme' (Pinheiro *et al.* 2013). '*Crassula helmsii* abundance' was set as the dependent variable and 'plot treatment' as the fixed factor, with 'survey number' included as a random factor to account for the repeated measures. The covariance structure 'corCAR1()' was specified in the model, because the repeated surveys had been conducted at unequally spaced time intervals (Field *et al*. 2012).

6.5. Results: Field experiment

At the first survey after set-up *C. hemsii* had colonised 26 experimental plots across a wide distribution of the experimental area, and this species continued to spread into and colonise adjacent plots throughout the experiment (Fig. 24). During the two years when surveying was conducted, *C. helmsii* was recorded to have occurred in 71 out of the 75 experimental plots. However *C. helmsii* did not persist in all of these plots; at the end of the experiment this species was recorded to occur in 59 plots, having disappeared from three control plots, three grass dominated plots, and six moss dominated plots.

Other plants were colonising and spreading across the experimental area alongside *C. helmsii*. The amount of co-occurring vegetation cover recorded in the bare ground plots increased over time, reaching a similar amount of cover as in the planted plots by summer 2012 (Fig. 25). The dominance of *A. stolonifera* and *S. cuspidatum* decreased within their respective plot treatments, reaching a similar and lower level of abundance in all treatments by the end of 2012 (Fig. 25). There was a particularly sharp decline in *A. stolonifera* and *S. cuspidatum* abundance between 14th June and 24th July 2012, which may have been caused by inundation of the plots in June 2012. Based on these changes in the plant community, the contrast in competitive pressure between the different plot treatments created at the start of the experiment, could only be seen to have persisted during the first year of the two-year experiment.





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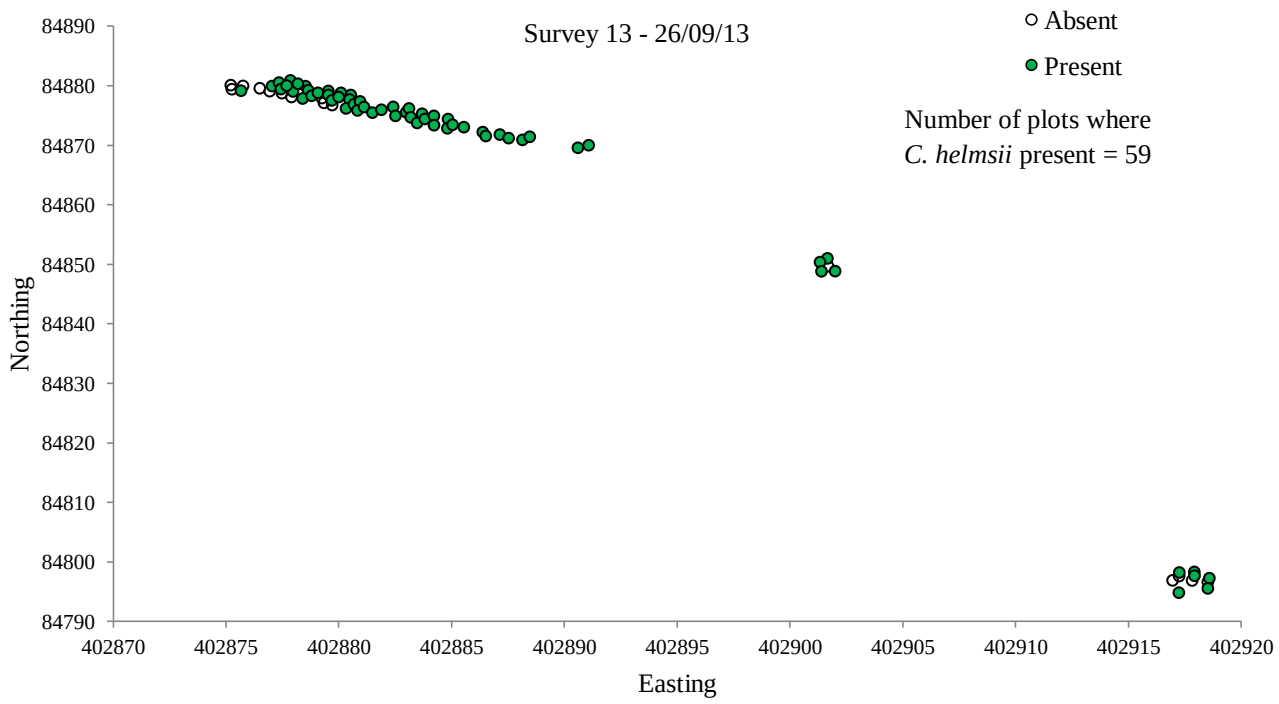
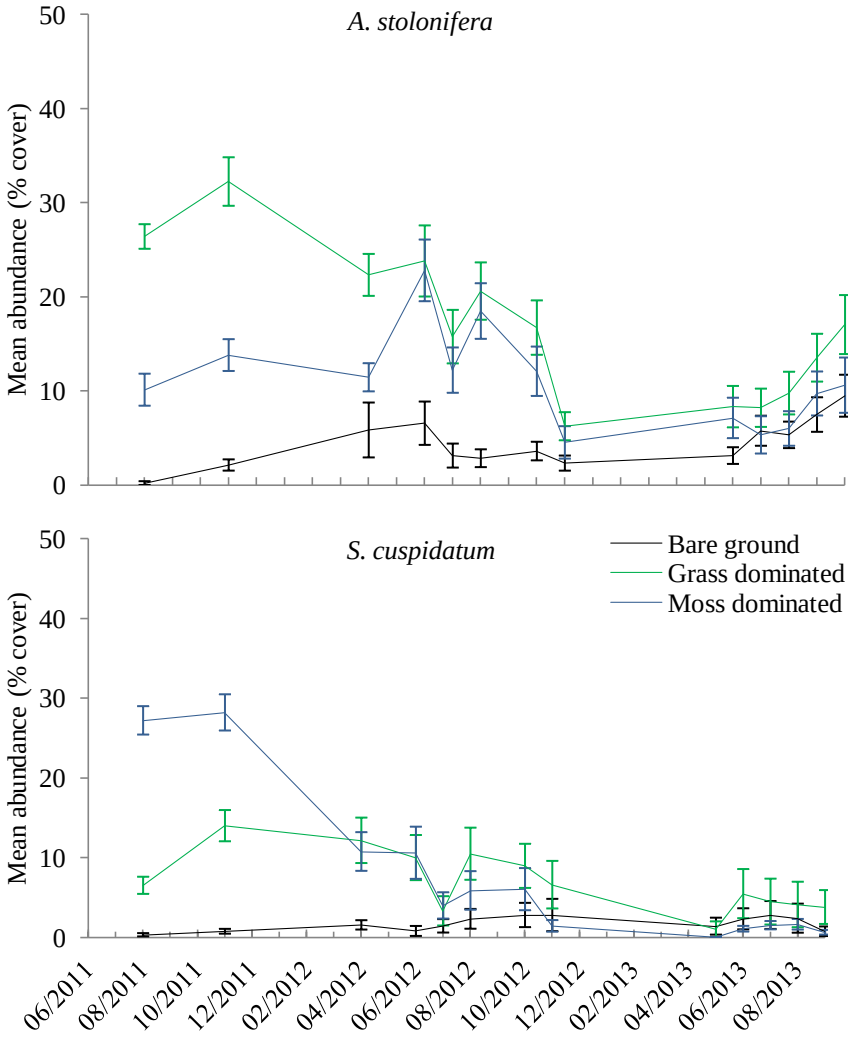


Figure 24 (and previous page). Each data point represents one of the 75 experimental plots. These plots are arranged on the graph based on their easting and northing coordinates (coordinate system: WGS 1984), providing an accurate representation of how the plots were arranged in space relative to one another. The data points coloured green represent plots where *Crassula helmsii* was recorded to occur, on the first (survey 1), middle (survey 6), and last (survey 13) surveys of the two-year experiment, and thus indicate the spread of *C. helmsii* across the experimental area over time.



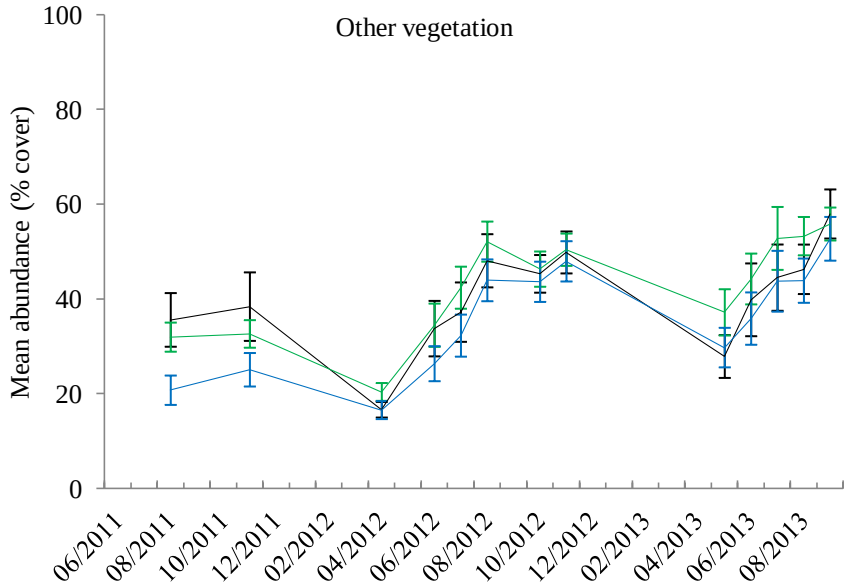


Figure 25. The mean abundance (± 1 SE) of vegetation recorded over two years, in three different planting treatments; 1) left as bare ground, 2) planted with grass dominated vegetation, or 3) planted with moss dominated vegetation. Data are shown for the abundance of *Agrostis stolonifera* the dominant grass species, *Sphagnum cuspidatum* the dominant moss species, and for the summed abundance of all other species excluding *Crassula helmsii*.

Does plot treatment have an effect on the speed of C. helmsii colonisation?

There was no significant difference between the plot treatments, regarding the median value of when *C. helmsii* was first recorded (H = 0.817, p = 0.665). Meaning that plot treatment did not have a significant effect on the time when *C. helmsii* first colonised a plot, and therefore that *C. helmsii* colonised plots of all three treatments at a similar speed. In all plot treatments, there tended to be a higher frequency of first occurrence within the earlier surveys (Fig. 26).

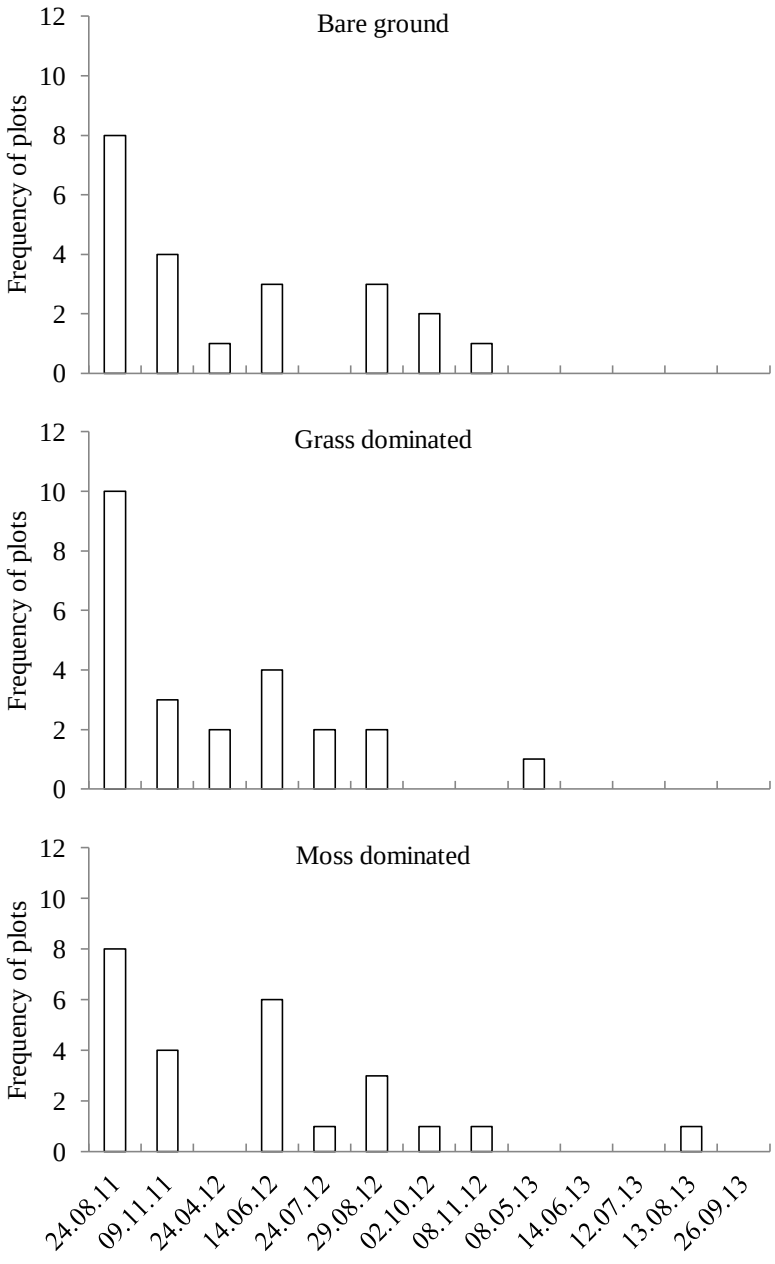


Figure 26. Each bar represents the frequency of experimental plots within a survey where *Crassula helmsii* was recorded for the first time; indicating recent colonisation. The X axis categories relate to each of the 13 repeated surveys, arranged in chronological order, and the data are split into three graphs to separately describe colonisation into (top) bare ground, (middle) grass dominated, and (bottom) moss dominated experimental treatments. There were 25 plots in each experimental treatment.

Does plot treatment have an effect on the abundance of C. helmsii?

There was no significant effect of plot treatment on the mean abundance of *C. helmsii* ($F_{(2, 960)}$ = 1.124, p = 0.325), and the increase in abundance over the course of the experiment was similar in plots of all three treatments (Fig. 27). *Crassula helmsii* did not come to dominate the plant community in any of the three plot treatments (Fig. 28).

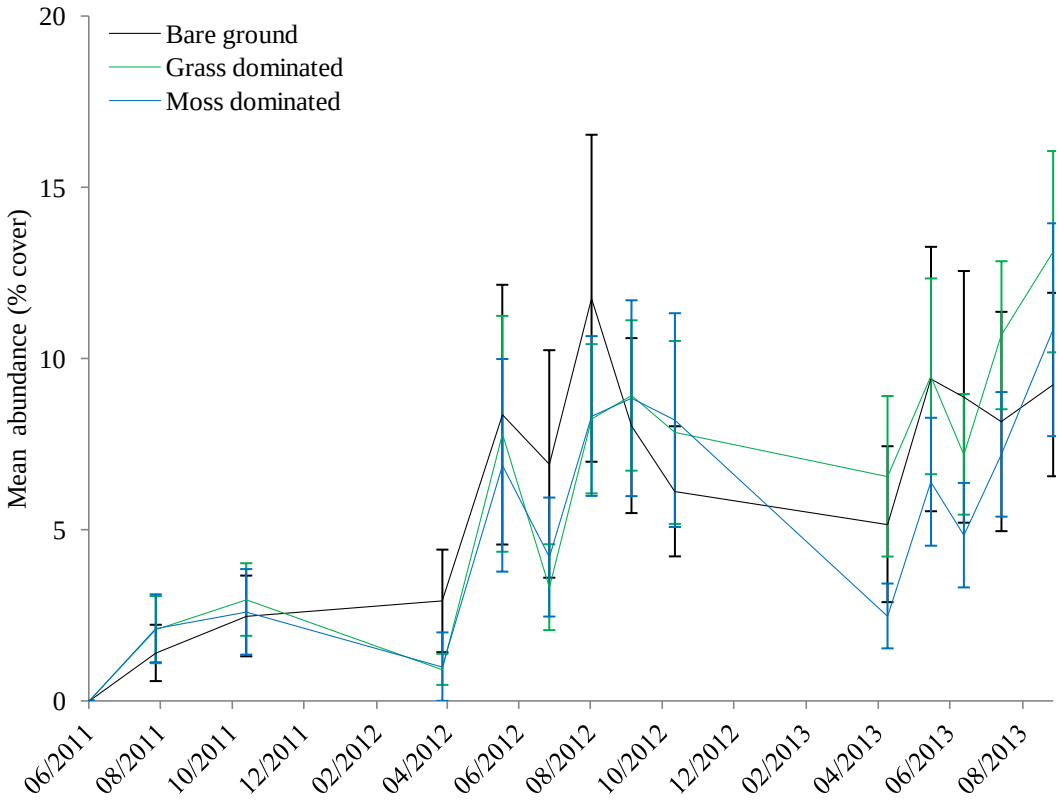


Figure 27. The mean abundance (± 1 SE) of *Crassula helmsii* recorded over two years, in three different planting treatments; 1) left as bare ground, 2) planted with grass dominated vegetation, or 3) planted with moss dominated vegetation.

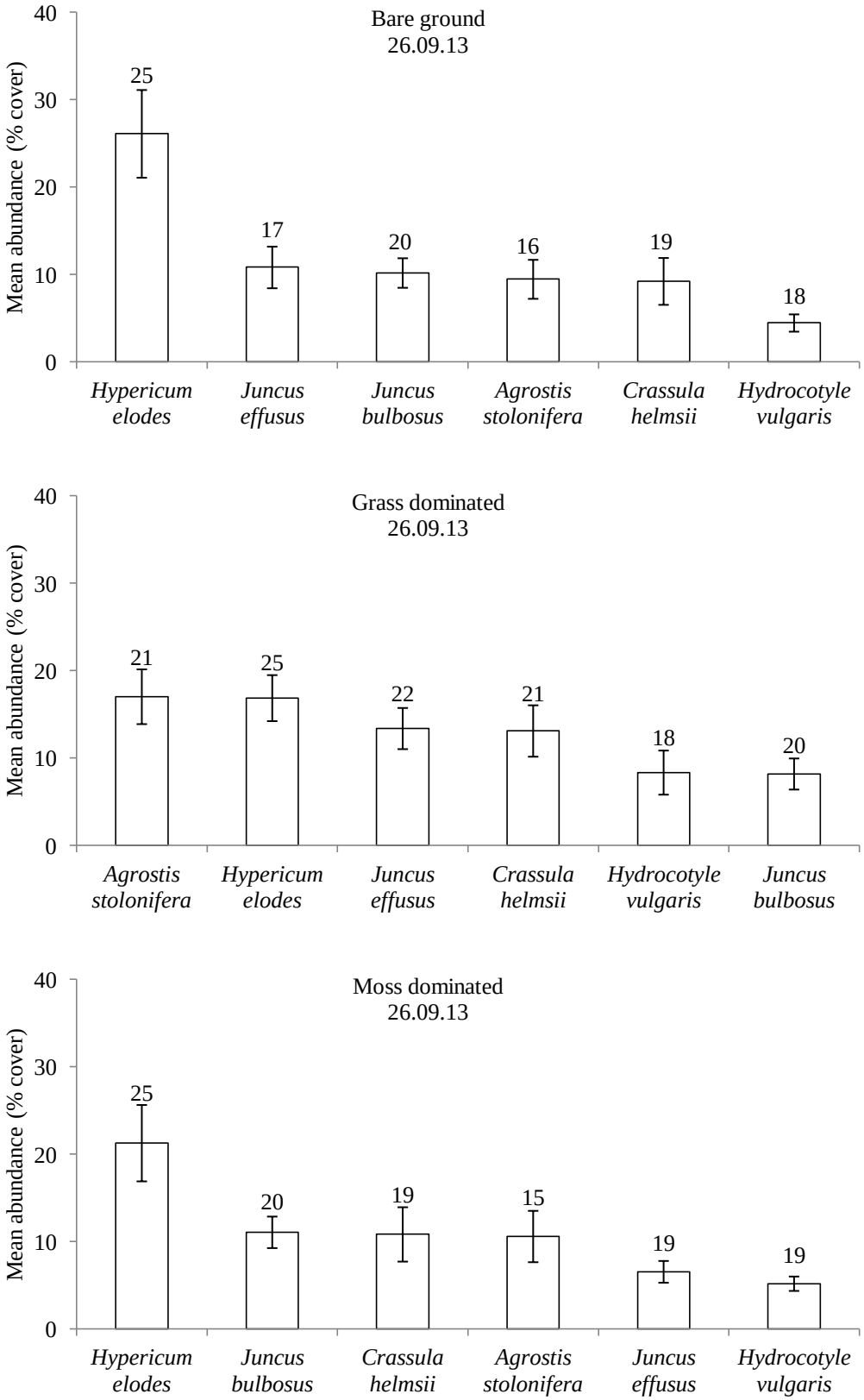


Figure 28. The mean abundance (± 1 SE) of the most abundant and frequently occurring species as recorded in the final survey of the experiment (26.09.13). The species shown are those which had a mean abundance of greater than 5 % cover. The number of plots in which each species was recorded is shown above the corresponding bar on the graph.

6.6. Discussion

6.6.1. Common garden experiment

The significantly higher expansion rate of the *C. helmsii* control compared to the native species controls, during the first growth phase, is reflective of the fact that *C. helmsii* was able to begin expansion in the weeks directly following planting and to rapidly increase in horizontal cover during this time period. In contrast, the native species showed little or no horizontal expansion during the same period. The timing of the common garden experiment is likely to have affected this result, by influencing the ability of the test species to respond to available bare ground at the start of the experiment, which was in September and thus at the end of the main growing season in the UK. It is possible that if the experiment had begun earlier in the growing season, the seasonally declining species *H. elodes* and *H. vulgaris* (Hill *et al*. 2004; Stace, 2010) may have shown a greater initial response. The results from this experiment did not find that *C. helmsii* had an intrinsically faster expansion rate than the natives, because during the second growth phase when all four species showed horizontal expansion, the results from the control treatments found that *C. helmsii* did not have a significantly higher expansion rate than *A. tenella* or *H. vulgaris*, and indeed had an expansion rate which was significantly lower than *H. elodes*. To separate the effects of innate response speed and season, additional experiments could be conducted which measure the response speed of the test species at different stages during the growing season, similarly to Barrat-Segretain & Amaros (1995) who compared the speed at which aquatic vegetation recovered after disturbance, in summer (July) and winter (December).

During the first growth phase, the expansion rate of *C. helmsii* was significantly lower in the control than when *C. helmsii* was paired with the native species. From observation of the cover over time graphs, it can be seen that this result was obtained because *C. helmsii* cover reached a plateau much sooner in the control than in the paired species treatments, and so when expansion was calculated over the whole of the first growth phase (five months), the slope value for the control was lower. In fact, the reason that expansion ceased much earlier in the control was because *C. helmsii* had expanded to occupy nearly all available bare ground by the end of the first month; both of the two plugs of *C. helmsii* vegetation had expanded to cover on average of 50 % of the total available space. In contrast, the native species which were paired with *C. helmsii* showed little expansion during the first growth phase and so the single plug of *C. helmsii* could continue on expanding much further across the space in the pot. In this first growth phase therefore, it can be concluded that interspecific competition from the native species was minimal and *C. helmsii* expansion was much more limited by intraspecific competition. In the second growth phase there was no significant difference in the expansion

rate of *C. helmsii* between the control and the paired-species treatments. By this stage in the experiment, the native species within the paired-species treatments were also showing expansion, and so the strength of interspecific competition may have been greater in this second growth phase, imposing greater limits on the expansion rate of *C. helmsii*. It should be noted that even in the second growth phase, the effect of interspecific competition was not significantly greater than intraspecific competition. The general negative trend of expansion in *C. helmsii* paired with *H. vulgaris*, suggests that this native species may have limited the expansion of *C. helmsii* to a greater extent than the other native species or *C. helmsii* itself, however further replication of this experiment would be required to confirm this result as there was considerable variation in the data for this treatment.

The expansion rate of *A. tenella* was significantly lower when paired with *C. helmsii* compared to the *A.tenella* control, suggesting that competition from *C. helmsii* limited the expansion rates of this native species. The results from this experiment also show that when *C. helmsii* was paired with *A. tenella* the proportional cover was consistently higher for *C. helmsii* than for the native. This gives further indication that *C. helmsii* was the dominant species and able to limit the growth of *A. tenella*. It is important to note that *A. tenella* was not totally excluded from any of the replicates during the course of the experiment, although it is possible that if the experimental treatment had been carried on then continued competition for resources may have lead to the species' decline.

The expansion rate of *H. elodes* was not found to be affected by *C. helmsii*, and the expansion rate of *H. vulgaris* was higher in the paired-species treatment compared to the control. Furthermore, the asymmetry in cover *C. helmsii* gained, during the first half of the experiment, did not prevent *H. elodes* and *H. vulgaris* from expanding rapidly during the second growth phase, and achieving a similar amount of cover to *C. helmsii* by the end of the experiment. Thus this experiment did not find evidence that *C. helmsii* was competitively dominant, compared to *H. elodes* and *H. vulgaris*. It is hypothesised here, that the height of the native species relative to *C. helmsii* may have influenced the competitive outcomes in this experiment; both *H. elodes* and *H. vulgaris* had a greater maximum height than *C. helmsii*, and so may have been able to compete by producing taller growth which overtopped the already present *C. helmsii* vegetation. This hypothesis is in line with Hejda *et al*. (2009), who reported that the height of non-native invasive plant species, relative to other competing species, was strongly associated with their level of impact on native plant communities; the greater the relative height of a non-native invasive plant, the greater the negative impact on species diversity.

6.6.2. Field experiment

The results of this experiment suggest that this species is adept at re-colonising cleared patches of ground. Indeed four weeks into the two-year experiment *C. helmsii* had appeared in 35 % of all plots. It is possible therefore, that there was no significant difference in the speed at which this species colonised the two planted treatments compared to the bare ground treatment, because *C. helmsii* had been able to disperse to and colonise the remaining bare ground within the planted plots, before the transplanted natives could spread out and occupy the space.

The creeping horizontal growth of *C. helmsii* was suggested in the introduction to this chapter (section 6.1.) to be an important mechanism for colonising bare ground. However in the case of this experiment, the scattered distribution of *C. helmsii* occupied plots recorded in the first survey suggests that many plots may have been separately colonised by individual vegetative propagules (Barrat-Segretain & Amoros, 1996), with creeping growth likely contributing to subsequent horizontal spread. The dispersal of clonal propagules has also been highlighted in other non-native invasive plants as a mechanism for extensive colonisation of an invaded area (Aguilera *et al.* 2010; Lin *et al*. 2012), and it is known that *C. helmsii* can disperse via this mechanism as well as by creeping horizontal growth (Dawson & Warman, 1987; EPPO, 2007).

In the field experiment, no significant difference was found in the abundance of *C. helmsii* between the plots which were initially bare ground, and the plots into which native vegetation was transplanted. The results showed that *C. helmsii* obtained a similar abundance within all three treatments, suggesting that manipulating the amount of competition at the start of the experiment did not have an effect on the abundance of this species. It is possible that this result reflects that *C. helmsii* was able to increase in abundance despite competition from native vegetation in the planted plots. Such an interaction however, would imply that the non-native invasive species was competitively stronger than the native species, as has been found in previous studies of non-native invasive-native plant community interactions (Flory & Clay, 2010; French, 2012). However, the data from the present experiment showed that *C. helmsii* did not dominate the plant community. Alternatively, this non-significant this result could indicate that over time the level of competitive pressure from native species equalised across the three treatments, thus imposing similar limits on *C. helmsii* abundance. There is more evidence to support this second hypothesis that the level of competitive pressure equalised, indeed data showed that the abundance of native vegetation occurring in each of the three treatments had converged by the mid-point in the experiment.

A major factor in the convergence of competitive pressure between the three plot treatments was likely to have been the growth of native species within the bare ground plots which could compete strongly against *C. helmsii*. To illustrate this, *H. elodes* was the most abundant species recorded in the bare ground plots at the end of the experiment and *H. vulgaris* was also relatively abundant. Indeed in a separate study of temperate wetland plants, Houlahan & Findlay

(2004) found that the native species chosen for the study were equally likely to be competitively dominant as the non-native species. Furthermore, germination of plants from the seed bank has been known to promote the development of the native plant communities following clearance disturbances (Jäger & Kowarik, 2010), and this could have been the case within the bare ground plots at Little Sea. The level of competitive pressure may also have equalised during the experiment, due to a reduction in the competitive pressure within the planted plots. In particular this may have occurred when the plots were inundated by lake water in June 2012 and in March 2013, because inundation can damage or uproot plants and can cause deposition of sediment layers (Bornette & Puijalon, 2011) resulting in a loss of vegetation cover.

It would be valuable to repeat the field experiment at a different site. Little Sea is characterised by low levels of plant available nutrients (JNCC, n.d) and the margins of the lake experience disturbance due to inundation and wave action (personal observation). It is possible that at another site with lower levels of inundation disturbance or higher nutrient levels, plant growth may have been less limited, thus allowing stronger competitive interactions to develop between species (Huston, 2004).

6.6.3. Is available bare ground an important pre-requisite to high *C. helmsii* abundance?

It was found that *C. helmsii* could respond to available bare ground ahead of native competitors, and that it quickly re-colonised bare ground in the field. As such, a faster response to available bare ground could allow to *C. helmsii* to grow and to utilise the resource before other species, and could thus lead to *C. helmsii* becoming proportionally more abundant in the developing plant community, as has been found for other non-native invasive species (Abraham *et al.* 2009; Dickson *et al*. 2012). Furthermore, *C. helmsii* showed a strong growth response in autumn when other species were no longer growing. This is another mechanism which has been described as facilitating the success of other non-native invasive plants (Wolkovich & Cleland, 2011), and which could also allow *C. helmsii* to spread into bare ground ahead of native competitors, allowing it to increase in abundance whilst other species are dormant or declining.

The results showed that the spread of *C. helmsii* was not strongly limited, despite the presence of native competitors. On the other hand *C. helmsii* was not found to totally exclude any of the native species in these experiments, and was not found to dominate the plant community. Based on these results therefore, it may be interpreted that competition from native species is unlikely to prevent *C. helmsii* spread or prevent this species from becoming relatively abundant within a plant community in the field, but the presence of strong native competitors could nevertheless impose some limits on abundance and prevent the invasive species from becoming dominant. As such, bare ground conditions which lack native competitors would very likely facilitate higher *C. helmsii* abundance.

7. Can the invasion of *C. helmsii* negatively affect the plant community in a *Phragmites australis* dominated fen habitat?

7.1. Introduction

A non-native invasive plant can achieve high abundance in plant communities where it is a stronger competitor than the resident native species, and in such cases, strong competition from the non-native can lead to the displacement of resident plants (Levine *et al*. 2003; Allred *et al*. 2010; Corbin & D'Antonio, 2010). A localised decline in plant abundance and diversity is therefore commonly highlighted as a negative impact caused by the invasion of a non-native plant (Vilà *et al*. 2011; Pyšek *et al*. 2012b). The magnitude of these reductions varies considerably however (Ricciardi & Cohen, 2007; Vilà *et al*. 2011; Pyšek *et al*. 2012b), and indeed there are examples where significant reductions have not been detected (Mills *et al*. 2009; Meffin *et al*. 2010). In part, this variation is related to the specificity of competitive outcomes between a particular non-native invasive plant and the resident species (Hejda *et al*. 2009; Thiele *et al*. 2010). Indeed some native resident species can be as strong or a stronger competitor, and provide biotic resistance against community dominance by the non-native invasive species (Levine *et al*. 2004; DeWine & Cooper, 2008; Meyer *et al*. 2010). Other species however, may be particularly vulnerable to displacement because they are unable to compete with the invader or because they are naturally scarce (Walck *et al*. 1999; Miller & Duncan, 2004; Thiele *et al*. 2010). Understanding which non-native invasive plants have the greatest impacts on biodiversity and which native species are more vulnerable to invasion is an over arching theme in invasion ecology (Davis, 2009), and also has a practical application for conservation by highlighting which communities and species most require protection from non-native invaders (Parker *et al*. 1999; Byers *et al*. 2002).

Because a decline in resident plants is a recognised indicator of an invasion impact, it is useful for preliminary research into the impacts of a non-native invader, to test communities for an inverse pattern between non-native abundance and resident plant abundance and diversity (Houlahan & Findlay, 2004; Gerber *et al*. 2008; Davies, 2011). If such patterns are detected, further research is justified to understand the mechanisms behind the relationship and whether the non-native invasive plant is the driver (HilleRisLambers *et al*. 2010; Murrell *et al*. 2011). Studies can look at the impacts on plants as classified taxonomically (Tererai *et al*. 2013) and plants as classified by their functional traits (Hejda, 2013). Moreover, it is valuable to investigate both species diversity and functional diversity for associations, because these two community measurements are not necessarily correlated (de Bello *et al*. 2006). Importantly, by investigation of exactly which species and functional traits decline with increasing non-native

invasive plant abundance, it may be possible to determine associations between the possession of certain traits and a negative response to invasion (Mason & French, 2008; Hejda, 2013), and so enable predictions to be made regarding what other potentially co-occurring species could be vulnerable (Drenovsky *et al*. 2012).

Non-native invasive plants may not be detected in the field until they are well established (Hulme, 2006), meaning that information relating to community change during the initial stages of an invasion may not be available for a field site, as described by D'Antonio *et al*. (1998). In such cases identifying differences between highly invaded locations and those which are little or un- invaded, is useful for inferring how the invasion process may have altered the plant community, and has thus been widely utilised to investigate the impacts of specific non-native invasive species on the abundance and diversity of resident plants (e.g. Hejda & Pyšek, 2006; Gerber *et al*. 2008; Hejda *et al*. 2009; Aguilera *et al*. 2010; Stiers *et al*. 2011). In this study, such field data were collected from across a range of *C. helmsii* abundance, which was located within *Phragmites australis* (common reed) dominated fen. This is a habitat in which the shallows and margins of freshwater lakes are managed to maintain stands of *P. australis*, interspersed with open patches occupied by a more diverse assemblage of herbaceous species. This type of habitat is considered beneficial for biodiversity, and in particular *P. australis* stands (reedbeds) provide shelter and feeding opportunities for species of wetland birds and their chicks (McBride *et al*. 2011). The displacement of *P. australis* by a non reedbed forming plant such as *C. helmsii* could have negative ecological impacts beyond the plant community. *P. australis* itself is a tall (≤ 3.5 m), perennial graminoid, which forms dense reedbeds in fresh and brackish water habitats, growing marginally or as an emergent (Rose, 1989; Hill *et al.* 2004). It is a native community dominant in UK freshwater habitats (Rodwell, 1998) and a highly competitive and invasive species in North America (Chambers *et al*. 1999; Ailstock *et al*. 2001).

The aim of the study was to investigate whether *C. helmsii* could achieve high abundance by displacing plants in the resident community, and in this way find evidence that the invasion of *C. helmsii* into *P. australis* dominated fen was having a negative impact on the resident plant assemblage. Evidence in this case was given to be negative correlations between *C. helmsii* abundance and variables of abundance and diversity in the resident plant community; patterns which are consistent with the displacement of species. Toward this aim comparisons were made between three elements of the plant community. Firstly, field surveyed data were analysed for a correlation between *C. helmsii* and *P. australis*, giving an indication of whether these two species were in competition and could potentially displace or suppress each other. Secondly, data were analysed for a correlation between *C. helmsii* and the subordinate herbaceous community, giving an indication of whether *C. helmsii* was displacing these species. Thirdly, the correlation between *C. helmsii* and the subordinate herbaceous community was compared to the correlation between *P. australis* and the subordinate herbaceous community, providing an

indication of the relative impact of *C. helmsii* in the community compared to a strongly competing native species. Correspondingly three questions were asked: 1) Is there evidence that *C. helmsii* is in competition with *P. australis*? 2) Is there evidence that *C. helmsii* has displaced plant species in the subordinate plant community? 3) Is the impact of *C. helmsii* on the subordinate plant community, greater than the impact of *P. australis* on the subordinate plant community?

7.2. Methods and materials

7.2.1. Data collection: plant species

Field survey data used in this chapter were collected from three 'sites' in Cambridgeshire, UK. These were: Fen Drayton, Kingfishers Bridge, and Ouse Fen. Within each of these sites, data were collected from sample locations of $1m^2$, which were located along the margins of lakes. Stands of *P. australis* occurred frequently and at high densities along these margins, and *C. helmsii* was also known to have invaded the margins. The sample locations were distributed in a stratified sampling design, and in total 51 sample locations were visited across the three sites. The methods by which these sampling locations were selected, is explained in detail in chapter two, section 2.1.

In each sample location the abundances of *C. helmsii* and *P. australis* were recorded as estimates of percentage cover. All other co-occurring plants were identified to species using standard keys for vascular plants (Rose, 1989; Rose, 2006), and the percentage cover was estimated separately for each species. The amount of bare ground, representing an absence of co-occurring vegetation, was also recorded as an estimate of percentage cover. Surveying of the plant community was carried out between 16th July and 23rd August 2012, and was conducted simultaneously with the collection of the abiotic data presented in chapter three.

7.2.2. Data collection: plant traits

All 40 co-occurring species recorded in the field survey were categorised based on a number of plant traits. Seven different trait categories were included in this analysis, within which were nested 28 traits (Table 9). The trait categories were chosen based on how a species occupies the space (morphology), when it occurs (seasonality), and how it spreads into new space (reproduction). Data relating to morphology, seasonality, and reproduction were chosen for this analysis as it was considered that such traits could play a role in determining how well a species could compete for space.

Trait data were sourced from online databases of plant species traits (Fitter & Peat, 1994; Hill *et al*. 2004; Kleyer *et al*. 2008), and from botanical keys (Rose, 1989, 2006). The databases were used as the primary source of trait information and provided the majority of the trait data for the species included in the analysis, whilst the floral keys were a secondary source and only referred to when information on a trait could not be found within the databases. A trait matrix was produced from these data, which listed the specific traits of each species, data on how frequently each species occurred, and the mean overall abundance of each species (appendix III).

Table 9. The plant trait categories used to group the species that were recorded in the field survey, and the traits nested within each category. For each category, an explanation is given of how species were categorised.

Category	Explanation of traits
Main reproductive strategy:	The most frequently utilised method of reproduction. Species were categorised as either utilising 'sexual', 'clonal', or 'sexual and clonal' strategies.
Primary clonal organs:	Whether a species produces clonal organs, and if so of what form. Species were categorised as primarily possessing vegetative 'fragments', aboveground 'stolons', or belowground 'rhizomes'.
Leaf persistence:	For how long a species maintains its leaves throughout the year. Species were categorised as either 'aestival', 'semi-evergreen', or 'evergreen'.
Typical maximum height:	The maximum height a species typically grows to. Species were classed as 'short' if they had a typical maximum height of 0.1 – 25cm, classed as 'medium' if they had a typical maximum height of 26 – 50 cm, classed as 'tall' if they had a typical maximum height of 51 - 100 cm, and classed as 'v.tall' if they had a typical maximum height of greater than 100 cm. Species were classed as 'floating' if their maximum height was dependent on the water depth.
Typical stem position:	The natural orientation of the main stem. Species were categorised as 'erect', 'prostrate', climbing', or 'floating'.
Typical leaf area:	The area to which the leaves of a particular species typically grow to. The system of categorisation used in the Ecoflora trait database (Fitter & Peat, 1994), which had four categories (0.1 – 1, 1 – 10, 10 – 100, and 100 – 1000 cm^2), was adapted so that leaves of 0.1 – 2 cm^2 were categorised as 'very small', leaves of 1 – 10 cm^2 as 'small', leaves of 10 – 100 cm2 as 'medium', and leaves of 100 – 1000 cm^2 as 'large'.
Leaf dimensions:	How wide compared to long the leaves a particular species are. The system of categorisation used in the Ecoflora trait database (Fitter & Peat, 1994), which had three categories (> 3x as long as wide, 1 – 3x as long as wide, and length = width), was adapted so that leaves > 3x as long as wide were categorised as 'narrow', leaves 1 – 3x as long as wide as 'broad', and leaves length = width as 'equal'.

7.2.3. Statistical analysis

Survey data from the three *C. helmsii* invaded sites (Fen Drayton, Kingfishers Bridge, and Ouse Fen) were analysed together as a single dataset. The similarity in the structure of the vegetation at these sites, which were dominated by *P. australis* with herbaceous mainly perennial subordinate species; and the relative geographic closeness of these sites, all falling within approximately 33 square miles of Cambridgeshire; was considered justification for combining these data to represent a single plant community. It was acknowledged however, that there was between-site variation in pool of subordinate species which were recorded in the survey. To assess the magnitude of this between-site variation in the plant community, a 2D non-metric MDS (Multi Dimensional Scaling) graph was plotted of the Bray-Curtis similarity between sample locations, based on species presence and absence. The graph was plotted using the program PRIMER 6 version 6.1.15 (© PRIMER-E Ltd, 2012), and was visually inspected for clustering of sample locations into sites.

Prior to analysis all percentage cover data were transformed using an arcsine transformation (see chapter two, section 2.2). For each $1m^2$ sample location, three variables were calculated which related to plant species abundance and diversity of the co-occurring plant community: 1) total co-occurring plant abundance, 2) species diversity, and 3) species evenness). The total abundance of the co-occurring plant community was calculated as the summed percentage cover of all co-occurring plant species, species diversity was calculated using the Shannon-Weiner diversity index (H), and species evenness was calculated using the Shannon-Wiener Index of species evenness (J) (see chapter two, section 2.2). In all cases these variables were calculated excluding *C. helmsii* and *P. australis*, thus obtaining results regarding the properties of the communities co-occurring with these dominating species.

For each $1m^2$ sample location, three variables were also calculated which related to plant trait abundance and diversity within the co-occurring plant community: 1) functional richness, 2) functional evenness, and 3) functional dispersion. Functional richness is a measure of the extent of all traits possessed by species within a community (Villéger *et al*. 2008). Because the data for this study were all categorical, the functional richness index in this case specifically related to how many different combinations of traits occurred within a sample location (Laliberté & Shipley, 2011). Functional evenness is a measure of how evenly species differ in terms of their traits, and how evenly they differ in terms of their relative abundance within a community (Villéger *et al*. 2008). Functional dispersion, is a measure of the extent to which species differ in terms of their traits, from the typical suite of traits (centroid) within a community, and also takes into account the relative abundances of each species (Laliberté & Legendre, 2010). These indices were chosen because the methods, by which they were calculated, did not require species to initially be placed into functional groupings, but instead calculated functional

diversity based on the differences in the traits of each species to every other species (Villéger *et al*. 2008). Furthermore, calculations of these indices were compatible with categorical data (Laliberté & Shipley, 2011). Values for functional richness, functional evenness, and functional dispersion were calculated within the R package 'FD' (Laliberté & Shipley, 2011). This package required two separate data frames, with one containing the listed traits for each species included in the analysis and the other containing data on the abundance of each species in each sample location. R then calculated the corresponding indices of functional diversity using these data. Again, *C. helmsii* and *P. australis* were excluded from these data frames, providing functional diversity values for the plant community co-occurring with these two species.

Is there evidence that C. helmsii is in competition with P. australis?

The variable for *C. helmsii* abundance and the variable for *P. australis* abundance were analysed for a correlation using a Spearman rank test, and a scatter plot of *C. helmsii* abundance against *P. australis* abundance was inspected to assess the linearity of the relationship. The R package 'ppcor' (Kim, 2011) was used to conduct Spearman partial correlation tests, in order to investigate whether there was an association between *C. helmsii* and *P. australis* after taking into account shared variation associated with each of the co-occurring plant community variables: total co-occurring plant abundance, bare ground, species diversity, species evenness, functional richness, functional evenness, and functional dispersion.

Is there evidence that C. helmsii has displaced plant species in the subordinate plant community?

Analyses were conducted which tested for a negative correlation between *C. helmsii* and community composition. A number of Spearman partial correlation tests were run, which controlled for variation associated with *P. australis* abundance. Firstly, correlation tests were run between the variable for *C. helmsii* abundance and each calculated variable of the co-occurring plant community: total co-occurring plant abundance, bare ground, species diversity, species evenness, functional richness, functional evenness, and functional dispersion. Again, scatter plots of *C. helmsii* abundance against each variable, were inspected to assess the linearity of the relationships. Secondly, Spearman partial correlation tests were run between *C. helmsii* abundance and the abundances of individual recorded species. Species were only included in the analysis if they were recorded to have occurred in at least five sample locations, a total of 12 species, with a separate test run for each species against *C. helmsii*. Thirdly, Spearman partial correlation tests were run between *C. helmsii* abundance and the abundance of individual plant traits. For each trait, a value of abundance was obtained for each of the 51 sample locations, calculated as the summed percentage cover of all species possessing the trait. From these data

23 (out of 28) traits were selected for statistical analysis, based on the criteria that they occurred in at least five sample locations and were represented by at least two recorded plant species.

Is the impact of C. helmsii on the subordinate plant community, greater than the impact of P. australis on the subordinate plant community?

Spearman partial correlation tests were run, repeating the structure as outlined in the above paragraph. In this case *P. australis* abundance was tested for a correlation with each of the seven community variables, the individual species variables, and individual trait variables, whilst controlling for variation associated with *C. helmsii* abundance. The results obtained from *P. australis* were then compared to the results obtained from *C. helmsii*, indicating which species imposed greater competitive impacts.

7.3. Results

A total of 42 plant species were recorded over the three sites, of which 14 species had an overall mean cover of greater than 1 % (Fig. 29). The mean abundances of *P. australis* and *C. helmsii* were considerably higher than that of all other recorded plant species, and *P. australis* had a higher frequency (43 out of 51 sample locations) than *C. helmsii* (33 out of 51 sample locations). The plant community contained a high abundance of species which were capable of clonal reproduction, and which were tall with an erect stem, or floating (Fig. 30). Inspection of the 2D MDS plot (Fig. 31), indicating the Bray-Curtis similarity between sample locations based on species presence and absence, showed that the sample locations were not discreetly clustered based on site. Whilst a number of the sample locations from Fen Drayton and a number of the sample locations from Ouse Fen were differentiated from other sample locations, there were also differentiated clusters which contained sample locations from all three sites. Overall there was overlap in species composition in the sample locations from the three sites.

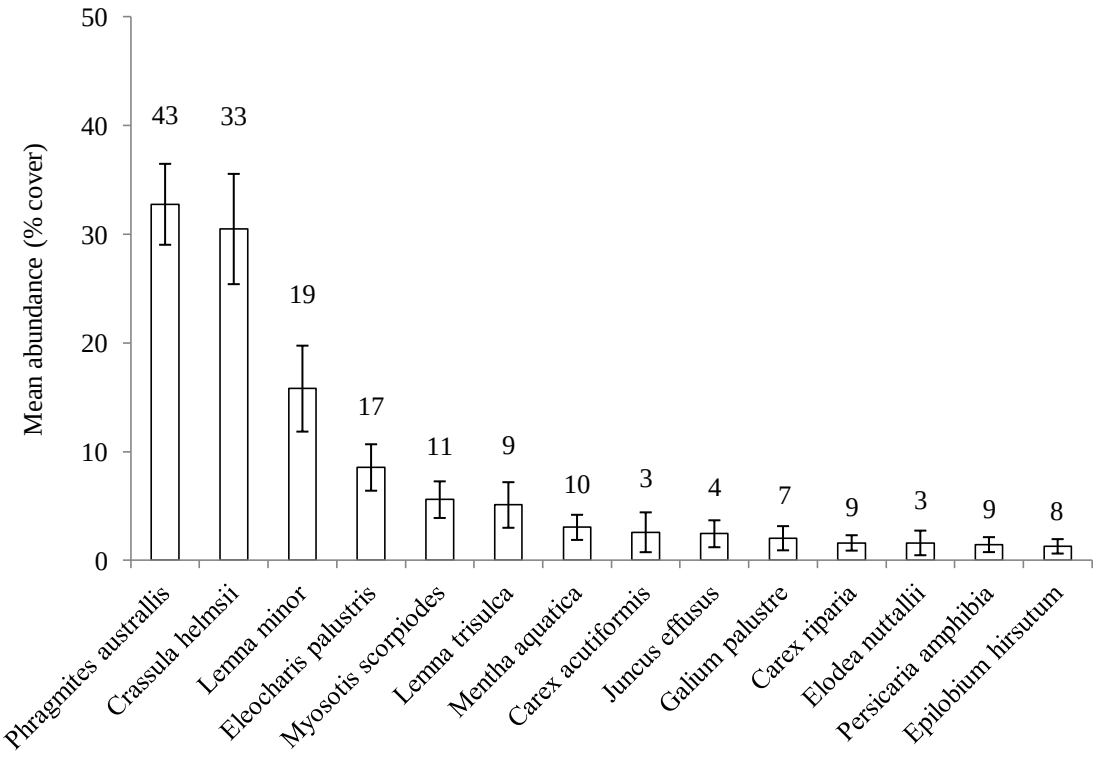


Figure 29. The plant species recorded in the field survey which had an overall mean abundance of greater than 1% cover. The bars show the mean abundance (± 1 SE) and the numbers above each bar show the frequency of sample locations within which each species was recorded.

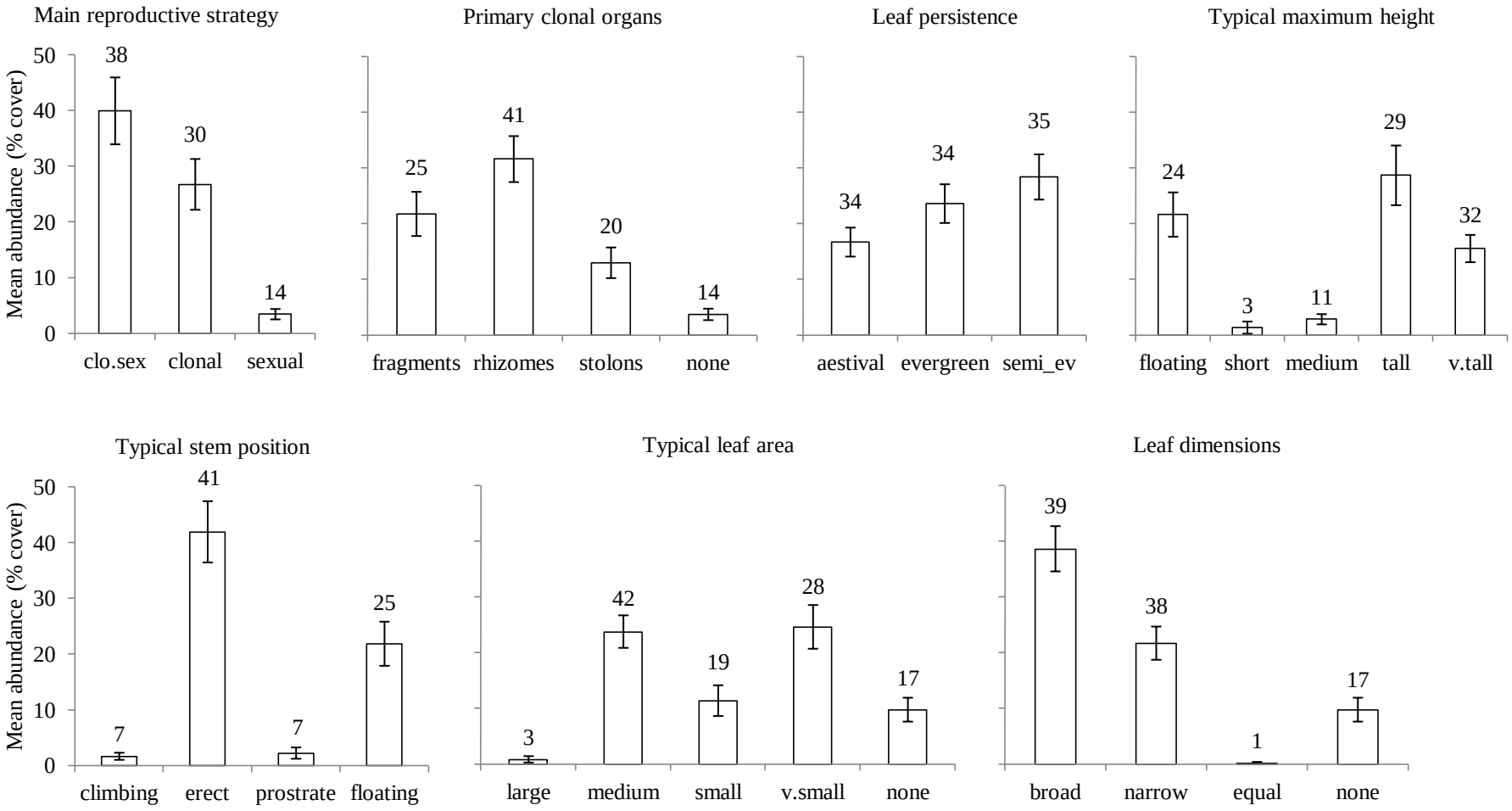


Figure 30. The overall mean abundance (± 1 SE) of plants possessing 28 individual plant traits, nested within seven trait groups. The numbers above each bar indicate the frequency of sample locations in which plants with each trait occurred. These data represent all 42 plants species that were recorded in the study.

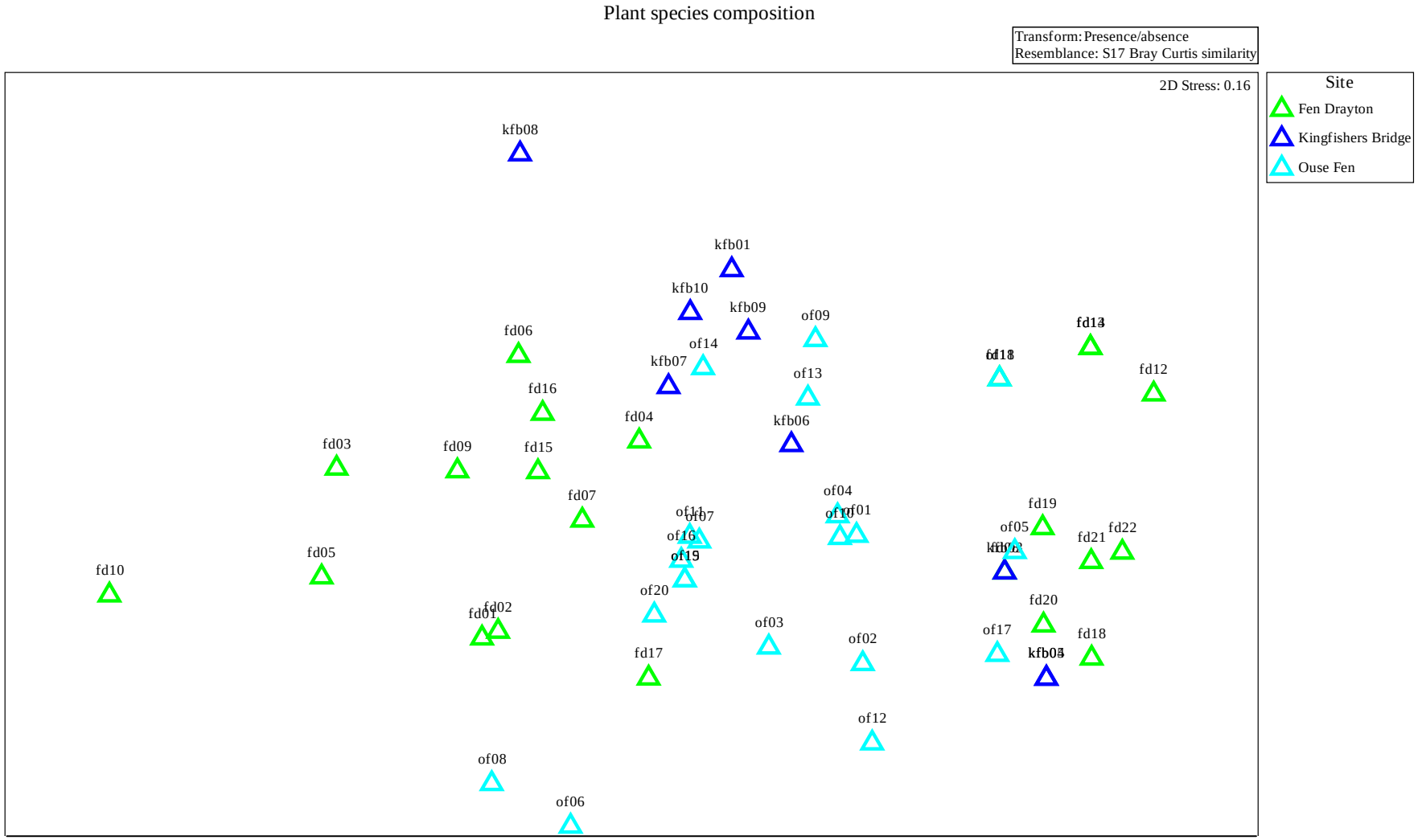


Figure 31. A 2D MDS (Multi Dimensional Scaling) plot representing each of the 51 sample locations included in the survey. The Bray-Curtis similarity between each sample location, based on the presence and absence of plant species, is represented by the distance between each plotted sample location point.

Is there evidence that C. helmsii is in competition with P. australis?

Crassula helmsii abundance and *P. australis* abundance was significantly negatively correlated (r_s = -0.311, df = 48, p = 0.026). This correlation was significant when a Spearman partial correlation accounted for variation in; the amount of co-occurring vegetation (r_s = -0.351, df = 48, p = 0.009), species diversity (r_s = -0.324, df = 48, p = 0.018), species evenness (r_s = -0.332, df = 48, p = 0.015), functional richness (r_s = -0.280, df = 48, p = 0.044), functional evenness (r_s = -0.295, df = 48, p = 0.033), and functional dispersion (r_s = -0.312, df = 48, p = 0.023).

Crassula helmsii and *P. australis* were not significantly negatively correlated when a Spearman partial correlation accounted for variation in the amount of bare ground (r_s = -0.156, df = 49, p = 0.273). Inspection of the scatter plot of *C. helmsii* abundance against *P. australis* showed that where *C. helmsii* was absent (0 % cover) there was wide variation in the abundance of *P. australis*. Where *P. australis* was absent there was wide variation in the abundance of *C. helmsii*, however *C. helmsii* abundance tended to be highest where *P. australis* abundance was below 40 % cover (Fig. 32).

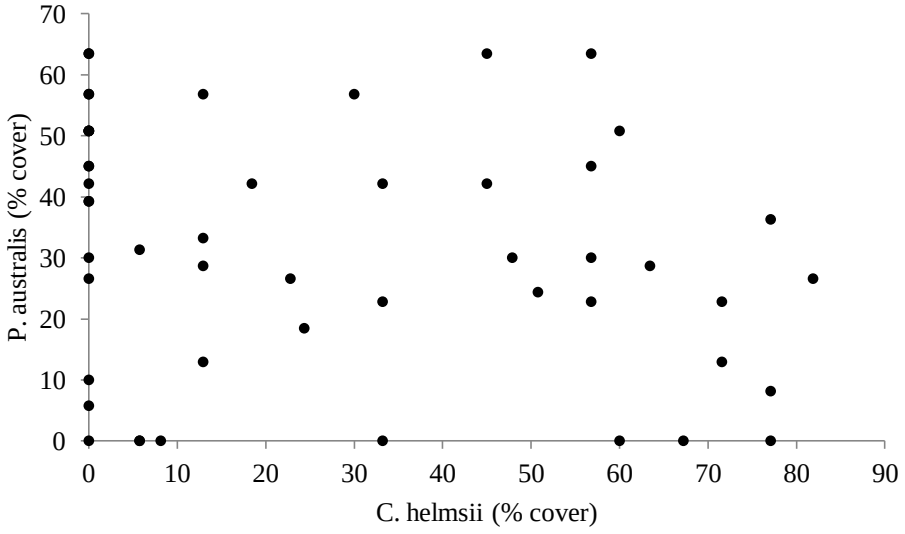


Figure 32. The abundance of *Crassula helmsii* plotted against the abundance of *Phragmites australis*. Data were obtained from 51 1m^2 quadrats, located within *P. australis* dominated fen habitat in Cambridgeshire, UK. When fit to a linear model, these species were found to be significantly negatively correlated (r_s = -0.311, df = 48, p = 0.026).

Is there evidence that C. helmsii has displaced plant species in the subordinate plant community?

Spearman partial correlation tests, accounting for *P. australis* abundance, indicated that the abundance of *C. helmsii* was strongly significantly negatively correlated with the amount of bare ground (r_s = -0.738, df = 48, p < 0.001). The abundance of *C. helmsii* was not found to be significantly correlated with the abundance of co-occurring vegetation (r_s = -0.230, df = 48, p = 0.101), species diversity (r_s = -0.097, df = 48, p = 0.498), species evenness (r_s = -0.128, df = 48, p = 0.371), functional richness (r_s = 0.048, df = 48, p = 0.739), functional evenness (r_s = 0.041, df = 48, p = 0.776), or functional dispersion (r_s = -0.030, df = 48, p = 0.837).

Inspection of the scatter plot of *C. helmsii* abundance against the abundance of co-occurring vegetation (Fig. 33) showed that there was considerable variation in the amount of co-occurring vegetation cover in sample locations where *C. helmsii* was absent or present at a low abundance. However, the abundance of co-occurring vegetation tended to be consistently lower in sample locations where *C. helmsii* abundance was highest. The same pattern was observed in the scatter plot of *C. helmsii* abundance against species diversity, and in the scatter plots of *C. helmsii* abundance against functional richness (Fig. 33).

Partial correlation tests against individual species found that *C. helmsii* was significantly negatively correlated with *Calystegia sepium* (r_s = -0.344, df = 48, p = 0.011), and *Lemna trisulca* (r_s = -0.345, df = 48, p = 0.011). *Crassula helmsii* was found to be significantly positively correlated with *Cirsium palustre* (r_s = 0.311, df = 48, p = 0.024) and *Epilobium hirsutum* (r_s = 0.365, df = 48, p = 0.007). Partial correlation tests against individual traits found that *C. helmsii* was significantly negatively correlated with seven traits (Table 10), and that *C. helmsii* was significantly positively correlated with sexual reproductive strategy (r_s = 0.398, df = 48, p = 0.003).

Table 10. The significant a) negative and b) positive results of Spearman partial correlation tests between *Crassula helmsii* and the abundance of individual plant traits, controlling for variation in *Phragmites australis* abundance.

	Trait group	Trait	Spearman partial correlation		
			r_s	df	p
a.	Main reproductive strategy	clonal	-0.356	48	0.008
	Primary clonal organs	fragments	-0.395	48	0.003
	Typical maximum height	floating	-0.427	48	0.001
	Typical stem position	climbing	-0.300	48	0.029
		floating	-0.410	48	0.002
	Typical leaf area	v.small	-0.448	48	0.001
	Leaf dimensions	broad	-0.297	48	0.031
b.	Main reproductive strategy	sexual	0.398	48	0.003

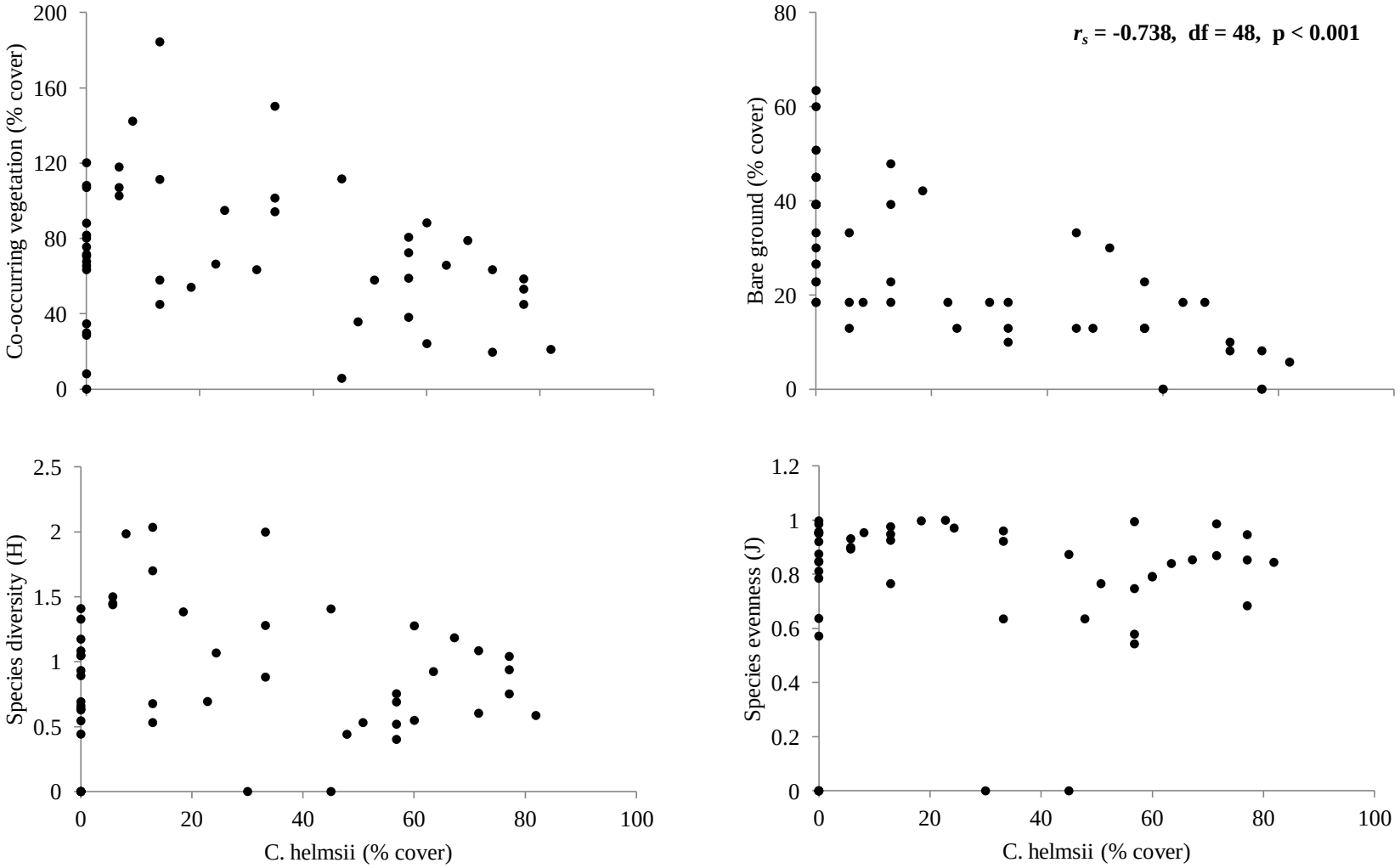
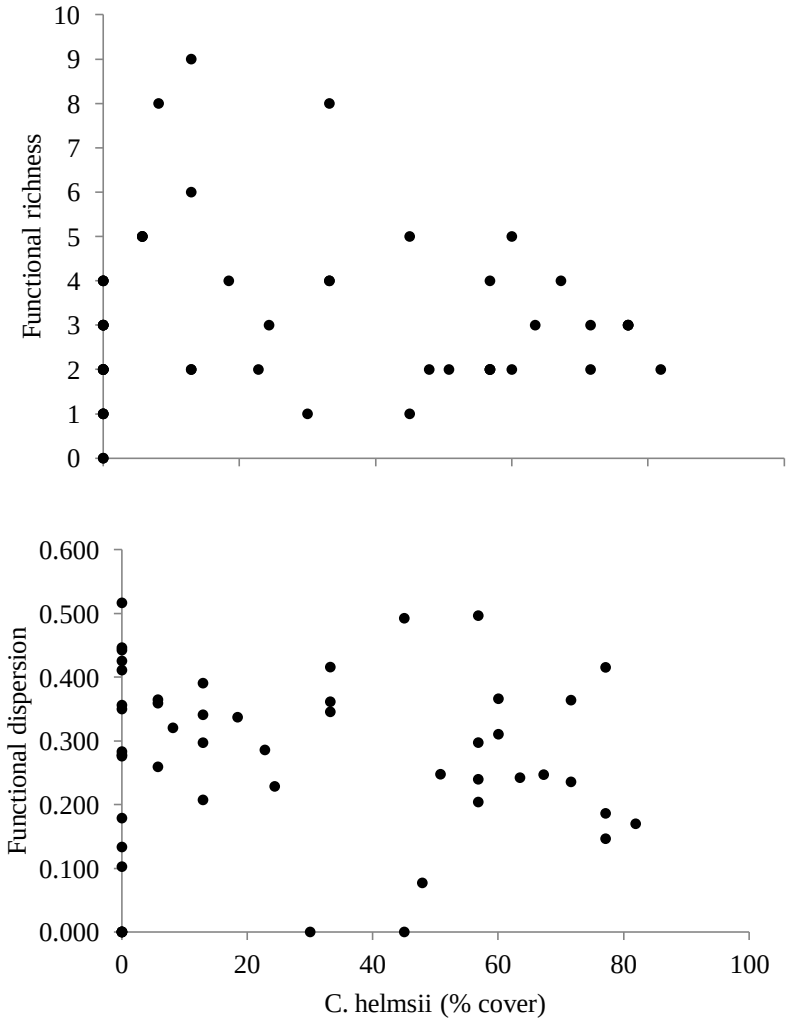


Figure 33. Continued on next page.



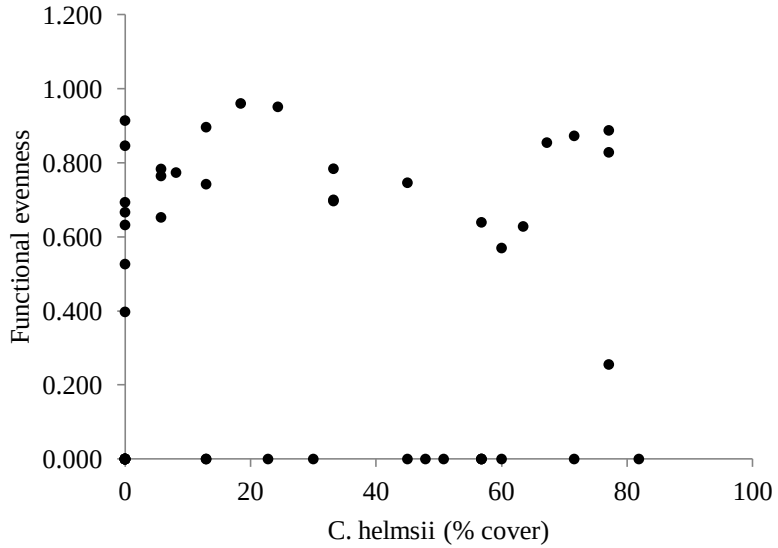


Figure 33 (and previous page). The relationships between *Crassula helmsii* abundance and seven variables of the plant community: the abundance of co-occurring (subordinate) vegetation, the amount of bare ground (absence of vegetation), species diversity (Shannon-Weiner H), species evenness (Shannon-Weiner J), functional richness, functional evenness, and functional dispersion. A significant strong negative relationship is indicated between *C. helmsii* and bare ground percentage cover (r_s = -0.738, df = 48, p < 0.001).

Is the impact of C. helmsii on the subordinate plant community, greater than the impact of P. australis on the subordinate plant community?

In contrast with *C. helmsii*, Spearman partial correlation tests (controlling for *C. helmsii* abundance) found that the abundance of *P. australis* was significantly negatively correlated with species diversity (r_s = -0.350, df = 48, p = 0.010) and functional richness (r_s = -0.313, df = 48, p = 0.022), and that *P. australis* abundance was not significantly correlated with bare ground (r_s = 0.071, df = 48, p = 0.620). Similarly to *C. helmsii*, the abundance of *P. australis* was not significantly correlated with the abundance of co-occurring vegetation (r_s = -0.252, df = 48, p = 0.071), species evenness (r_s = -0.278, df = 48, p = 0.055), functional evenness (r = -0.212, df = 48, p = 0.133), or with functional dispersion (r_s = -0.114, df = 48, p = 0.425).

In contrast to the *C. helmsii* scatter plots, the abundance of co-occurring vegetation, species diversity, and functional richness, did not show a clustering of low values in the sample locations where *P. australis* abundance was highest. Instead the scatter plots showed a pattern where species diversity and functional richness tended to be relatively high in the sample locations where *P. australis* was absent (0 % cover) (Fig. 34).

Inspection of the individual species Spearman partial correlations, showed that *P. australis* was significantly positively correlated with two species, and significantly negatively correlated with two species (Table 11), as was the case for *C. helmsii*. *P. australis* was significantly positively correlated with *Calystegia sepium* (r_s = 0.274, df = 48, p = 0.048), whilst *C. helmsii* had been significantly negatively correlated with *C. sepium*. Otherwise, *P. australis* was not significantly correlated with the same species as *C. helmsii* (Table 11). *P. australis* was found to be significantly positively correlated with five individual traits, with which *C. helmsii* had been significantly negatively correlated (Table 12). *P. australis* was also significantly negatively correlated with seven other individual plants traits, with which *C. helmsii* was not significantly correlated (Table 12).

Table 11. The results of Spearman partial correlation tests, conducted on plant abundance data (percentage cover). Shown are the outcomes of tests in which individual species in the plant community were tested against *Crassula helmsii* and *Phragmites australis*. The significant positive (grey) and negative (blue) correlation results are highlighted.

Species	*C. helmsii* (controlling for *P. australis*)			*P. australis* (controlling for *C. helmsii*)		
	r_s	df	p	r_s	df	p
C. sepium	-0.344	48	0.011	0.274	48	0.048
C. riparia	-0.064	48	0.657	0.199	48	0.159
C. palustre	0.311	48	0.024	-0.054	48	0.709
E. palustris	0.195	48	0.169	-0.267	48	0.055
E. hirsutum	0.365	48	0.007	0.131	48	0.361
G. palustre	-0.060	48	0.676	-0.125	48	0.382
L. minor	-0.269	48	0.053	0.335	48	0.014
L. trisulca	-0.345	48	0.011	0.022	48	0.877
M. aquatica	0.234	48	0.096	-0.271	48	0.051
M. scorpiodes	-0.226	48	0.108	-0.3988	48	0.003
P. amphibia	-0.157	48	0.272	-0.450	48	< 0.001
R. nasturtium aquaticum	-0.051	48	0.725	-0.061	48	0.673

Table 12. The results of Spearman partial correlation tests. Shown here are the outcomes for tests in which individual trait variables were tested against *Crassula helmsii* abundance, and against *Phragmites australis* abundance. Each trait variable represented the summed abundance of all species possessing that trait within each sample location. The significant positive (grey) and negative (blue) correlation results are highlighted.

Category	Trait	*C. helmsii* (controlling for *P. australis*)			*P. australis* (controlling for *C. helmsii*)		
		r_s	df	p	r_s	df	p
Typical reproductive strat.	clo.sex	0.217	48	0.123	-0.368	48	0.006
	clonal	-0.356	48	0.008	0.262	48	0.060
	sexual	0.398	48	0.003	-0.155	48	0.278
Primary clonal organs	fragments	-0.395	48	0.003	0.306	48	0.026
	rhizomes	0.221	48	0.117	-0.404	48	0.002
	stolons	-0.079	48	0.582	-0.306	48	0.026
	none	0.234	48	0.096	-0.259	48	0.064
Leaf persistence	aestival	0.197	48	0.163	-0.273	48	0.049
	semi_ev	-0.264	48	0.058	0.181	48	0.202
	evergreen	-0.100	48	0.487	-0.304	48	0.027
Typical maximum height	floating	-0.427	48	0.001	0.306	48	0.026
	medium	0.098	48	0.496	-0.228	48	0.104
	tall	0.247	48	0.077	-0.522	48	< 0.001
	v.tall	0.127	48	0.376	0.071	48	0.622
Typical stem position	climbing	-0.300	48	0.029	0.346	48	0.011
	erect	0.258	48	0.064	-0.480	48	< 0.001
	floating	-0.410	48	0.002	0.277	48	0.046
	prostrate	-0.078	48	0.586	-0.102	48	0.477
Typical leaf area	medium	0.110	48	0.444	-0.334	48	0.014
	small	0.052	48	0.717	-0.457	48	< 0.001
	v.small	-0.448	48	0.001	0.298	48	0.031
Leaf dimensions	broad	-0.297	48	0.031	-0.070	48	0.627
	narrow	-0.006	48	0.966	-0.193	48	0.172

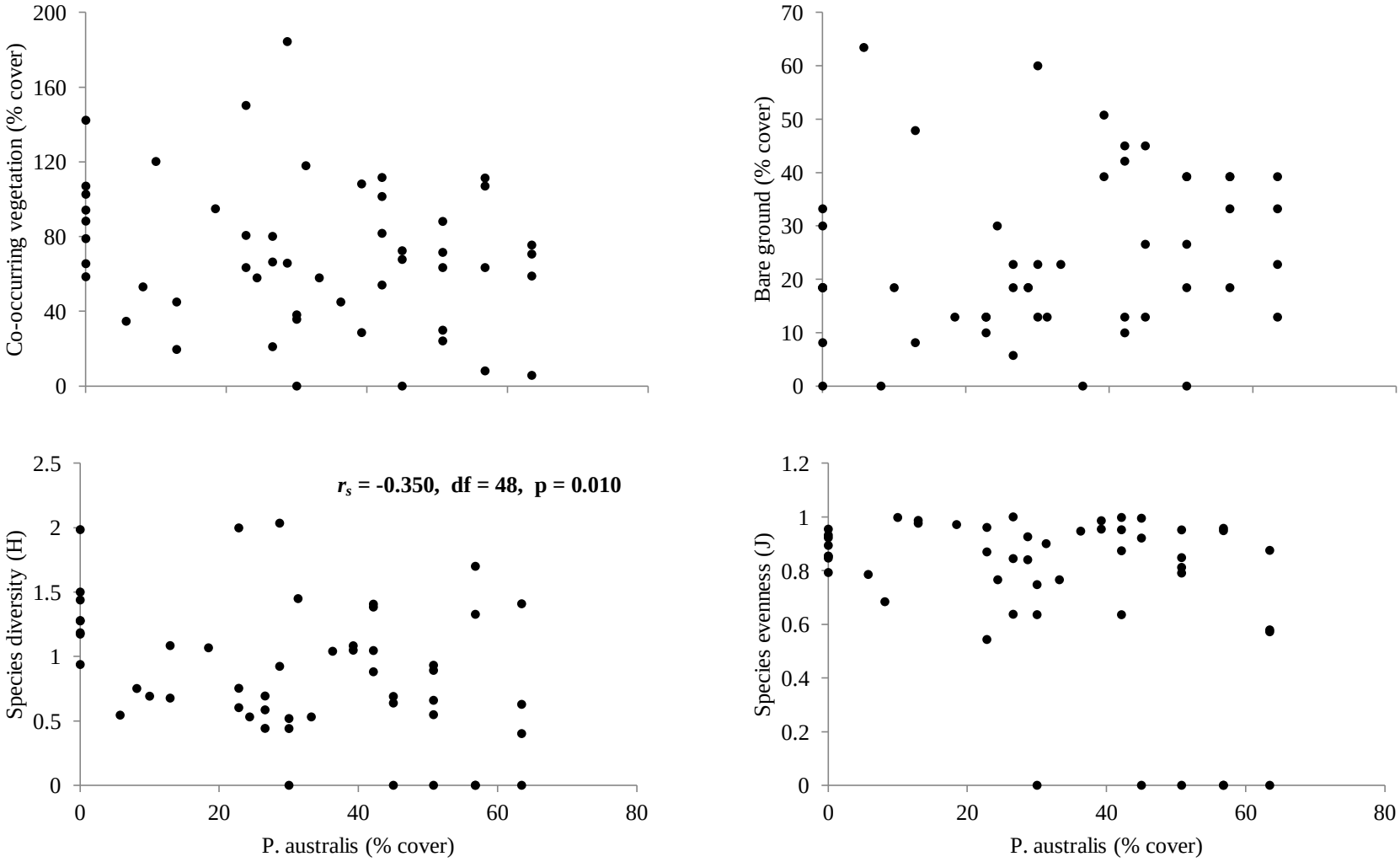
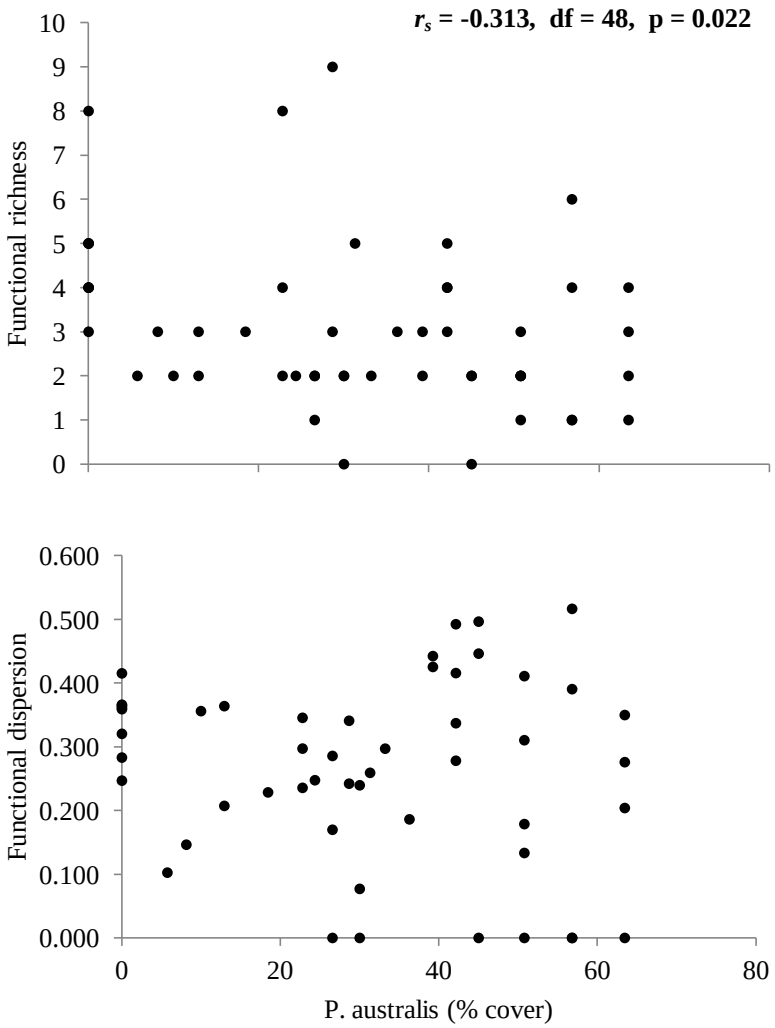


Figure 34. Continued on next page.



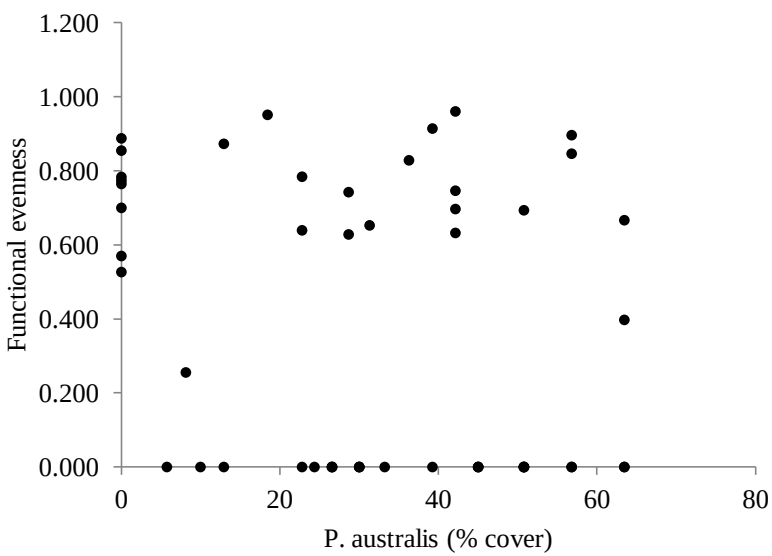


Figure 34 (and previous page). The relationships between *Phragmites australis* abundance and seven variables of the plant community: the abundance of co-occurring (subordinate) vegetation, the amount of bare ground (absence of vegetation), species diversity (Shannon-Weiner H), species evenness (Shannon-Weiner J), functional richness, functional evenness, and functional dispersion. Significant negative relationships were found between *P. australis* and species diversity (r_s = -0.350, df = 48, p = 0.010), and between *P. australis* and functional richness (r_s = -0.313, df = 48, p = 0.022).

7.4. Discussion

In this study *C. helmsii* and *P. australis* were found to be significantly negatively correlated, with a low to medium correlation coefficient value, in all but one of the seven partial correlation tests which were run. This indicates that there may have been suppression of abundance occurring between these species. In particular, *C. helmsii* abundance was highest where *P. australis* abundance was lower, which could signify that *C. helmsii* experienced reduced competitive pressure in these locations allowing it to achieve higher abundance, although it is also possible that where *C. helmsii* achieved very high abundance regeneration by *P. australis* was suppressed. The fact that these two species were not significantly correlated with each other when variation in the amount of bare ground was controlled for, suggests that the distribution of bare ground was a more important factor in structuring *C. helmsii* abundance, which is discussed in more detail below. Importantly, despite the significant negative correlation between *C. helmsii* and *P. australis*, these two species were nevertheless found to co-occur in many of the sample locations, and in some locations both achieving a cover of 50 % or greater. In these locations *C. helmsii* was seen to form a carpet at ground level whilst *P. australis* formed a canopy above. Therefore, it is concluded that whilst these species may experience some limitations in abundance due to competition with one another, overall evidence was found that these two species can coexist, both at relatively high abundances. To provide validation of this conclusion, it would be valuable to conduct repeat surveys of the sample locations, in order to investigate whether the abundance of *C. helmsii* compared to *P. australis* is stable over time, or whether one species increases in abundance in relation to the other. Controlled growth experiments could also be conducted to test the strength of competition between these two species.

Coexistence has been indicated in studies of other competitive native versus non-native invasive species. For example Meyer *et al*. (2010) compared the competitiveness of a vigorous North American native grass species (*Panicum virgatum*) to a non-native invasive grass species (*Miscanthus sinensis*), in a greenhouse experiment where the species where grown together at varying ratios. The authors found that although the native species grew comparatively larger, the growth of the non-native invasive species was not strongly suppressed, and it was concluded that these two species could co-occur in the field. The presently considered relationship, between *C. helmsii* and *P. australis*, is different from the one considered by Meyer *et al*. (2010) in that the present species differ more greatly in their morphology, and in particular stem position and maximum height. Indeed it is interesting how frequently *C. helmsii* was found growing extensively with *P. australis*, given how much taller *P. australis* was and thus how it could consistently restrict light availability to *C. helmsii*.

C. helmsii was not found to be significantly negatively correlated with the variables of the subordinate plant community; those relating to overall abundance, species diversity, and functional diversity. Thus these results did not provided a basis for evidence that *C. helmsii* invasion was having a negative impact on the subordinate plant community. This was in concurrence with Langdon *et al.* (2004), who found no significant change in plant species richness in ponds invaded by *C. helmsii* over a four to seven year period, although results were not provided for changes in species abundance over that time. Comparable results were also obtained by Mills *et al.* (2009), who found that although a non-native invasive shrub (*Rhamnus frangula*) had shown a substantial increase in cover between 1991 and 2006, there was not a significant change or decline in community composition during the time period. The authors discuss that this may be an example of a non-native invasive species coexisting with natives by inhabiting a slightly different niche. In the present study it should be noted however, that the statistical test used restricted analysis to the detection of linear relationships. As indicated in the results section, there may have been a pattern in which declines in the plant community were only evident in the sample locations where *C. helmsii* abundance was highest. It is possible that *C. helmsii* does have a negative relationship with the abundance and diversity of other plant species but only above a particular threshold of abundance. In line with this hypothesis, Gooden *et al.* (2009) demonstrated that the non-native invasive shrub, *Lantana camara*, had a considerably greater negative association with native plant species richness when it was above a particular percentage cover, which varied between approximately 30 % and 80 % cover depending on which native plant group it was being tested against. In particular therefore, further research on the effects of *C. helmsii* invasion, should focus on identifying whether such an impact threshold exists.

The strongest correlation result for *C. helmsii* was the significantly negative correlation with bare ground. This result is consistent with the hypothesis that *C. helmsii* is adept at filling bare space, because such a pattern could indicate that *C. helmsii* had increased in abundance by spreading out to occupy available bare ground. Indeed, colonising bare ground is equivalent to colonising an empty niche, and therefore also consistent with the hypothesis discussed by Mills *et al.* (2009), that a non-native invasive plant could increase in abundance without causing declines in native species diversity, if it were moving into an empty niche.

Whilst community-level correlations were not detected, *C. helmsii* was found to be significantly negatively correlated with two individual species (*Calystegia sepium* and *Lemna trisulca*). The negative correlation with *C. sepium* might have been related to the unsuitability of *C. helmsii* as structural support for this climbing species, or dense *C. helmsii* ground cover could have limited the ability of *C. sepium* to regenerate from its rhizomes. *L. trisulca* has small free-floating leaves, and so it is possible that emergent *C. helmsii* growth could have disrupted the dispersal of this species into heavily invaded areas. It has also been shown experimentally that *Lemna* sp.

growth can be suppressed by rooted plant species growing below, but specifically in conditions where access to nutrients is a limiting factor (Szabo *et al*. 2010). For both *C. sepium* and *L. trisulca* additional controlled experimentation would be required to confirm whether the detected relationship with *C. helmsii* was causal. *C. helmsii* was also significantly positively correlated with two species (*Cirsium palustre* and *Epilobium hirsutum*). It is surprising that *C. helmsii* was positively correlated with *E. hirsutum* given that the grazing exclosure experiment set up at Kingfishers Bridge (chapter five) showed an inverse relationship between the abundances of these two species. However, is possible that the positive correlation shown here is indicative that *E. hirsutum* was able to compete well in the plant community, in locations where *C. helmsii* was abundant. Indeed, both *C. palustre* and *E. hirsutum* are known to be strongly competing species, and *C. palustre* in particular grows well in recently disturbed habitats (Grime *et al*. 1995), similarly to *C. helmsii* (chapter six). Overall, that *C. helmsii* was significantly negatively correlated with only two species out of the 12 that were tested, and that *C. helmsii* was significantly positively correlated with as many species, does not provide strong evidence that *C. helmsii* had a negative impact on plant species in the subordinate plant community.

There appeared to be a strong link between the two species which were significantly negatively correlated with *C. helmsii,* and the individual traits which were significantly negatively correlated with *C. helmsii*; six out of seven of these individual traits were classified as being possessed by *L. trisulca*, and three traits as being possessed by *C. sepium*. However the correlation results were not identical, for example the correlation coefficient for *L. trisulca* and the correlation coefficient for floating stem position were different, and therefore the trait correlation results were unlikely to have been solely driven by the abundance of an individual species. In particular it may be valuable to conduct further research on whether *C. helmsii* has a negative impact on plant species which have a floating morphology, because as discussed above it is possible that emergent *C. helmsii* vegetation could alter the habitat structure in a way that could negatively affect such plants.

The impact of *C. helmsii* on the subordinate plant community was not found to be greater than the impact of *P. australis*. Indeed as discussed above, there was no strong evidence to support an impact of *C. helmsii* on the plant community. In contrast, *P. australis* was significantly negatively correlated with species diversity and functional richness, with this relationship in particular being driven by the more diverse communities in the sample locations where *P. australis* was absent. A negative correlation had been expected given the description of *P. australis* dominated habitat as reported in the introduction to this chapter; stands of *P. australis* interspersed with open patches occupied by a more diverse assemblage of herbaceous species (McBride *et al*. 2011). However, the correlation coefficients for the significant relationships with *P. australis* showed only medium effect sizes, and together with observations of the scatter

plots, the results indicate that there was much variation in the subordinate plant community which was not explained by variation in *P. australis* abundance. It was interesting to find that *P. australis* had the inverse correlative relationships with individual species and traits than *C. helmsii* did, as this pattern suggests divergent community associations between *P. australis* and *C. helmsii*, and therefore niche partitioning (Begon *et al*. 2002). As discussed above, *C. helmsii* could persist amongst the reedbeds but sample locations with the highest abundance were associated with the 'open' patches of the habitat, suggesting that these open patches were more optimal for *C. helmsii* growth.

The aim of this study was to investigate whether the invasion of *C. helmsii* into *P. australis* dominated fen could displace the resident plant assemblage. In summary, there was not strong evidence that this mechanism had occurred. However, because the plant assemblage which seemed to persist well alongside *C. helmsii* was the opposite of the assemblage that seemed to persist well alongside *P. australis*, it is possible that a situation in which *C. helmsii* and *P. australis* formed a stable coexistence, could be indicative of particularly species-poor plant community.

8. Main discussion

Summary of thesis results

The aim of this thesis was to better understand the mechanisms which allow *C. helmsii* to develop dense dominating vegetative mats. This aim was addressed firstly, by investigating how variation in the abiotic environment could affect *C. helmsii* abundance, using field data from several sites in southern and eastern England, in order to analyse how well variation in *C. helmsii* abundance could be predicted by variation in the pH, macronutrient levels, water depth, and shading of these sites. It was found that higher *C. helmsii* abundance was associated with lower levels of shade, but that *C. helmsii* still persisted even under conditions where overhead shading from vegetation was extensive. There was also found to be an association between *C. helmsii* abundance and pH. *Crassula helmsii* abundance was limited by pH within the acidic to neutral range, with abundance higher where pH was higher, but was not limited by pH within the neutral to alkaline range. It was concluded that knowledge of the levels of shading at a site, and the pH of the water, could contribute towards predictions of the extent of *C. helmsii* abundance. The inclusion of biotic variables within the linear model, for example the abundances of certain competitor plant species, may have accounted for some of the variation in *C. helmsii* abundance which was not accounted for by the abiotic variables assessed in this study.

Variation in the abiotic environment, and its effect on *C. helmsii*, was further investigated in the mesocosm experiment run to detect the limits of *C. helmsii* tolerance to salinity. This experiment found that the relative growth rate of *C. helmsii* was reduced in conditions of 2 and 4 ppt, compared to freshwater, and that the plant died in the 8 ppt treatment. These results therefore suggested that in the field *C. helmsii* growth, and therefore potentially its ability to develop dominating vegetative mats, would be reduced in brackish conditions up to 4 ppt, and that *C. helmsii* would be totally absent from conditions of 8 ppt or higher.

The effect of grazing on *C. helmsii* abundance, and specifically the effect of generalist vertebrate herbivores, was investigated by comparing a *C. helmsii* invaded plant community within fenced grazing exclosures to the community in adjacent unfenced plots. It was found that the abundance of *C. helmsii* declined in the fenced exclosure, compared to the unfenced plots, and that the abundance of tall competitive resident species was consistently higher in the fenced exclosure compared to the unfenced plots. These results therefore, indicated that grazing could facilitate a higher abundance of *C. helmsii* in situations where competitive resident plant species were selectively removed by grazers.

Two separate experiments were run to investigate how well *C. helmsii* could compete for bare ground, and thus how the availability of this resource in an ecosystem could affect the

likelihood of *C. helmsii* developing extensive vegetative mats. A common garden experiment was run to assess the rate and extent of expansion into bare space when *C. helmsii* was grown on its own compared to when grown paired with a native species, and to assess the rate and extent of expansion of *C. helmsii* compared to the native species. The results showed that *C. helmsii* began to expand much more quickly after planting than any of the three native species. This initial expansion phase occurred in September and October, thus also demonstrating that *C. helmsii* could continue expanding rapidly in autumn when other species had ceased growth. *Crassula helmsii* expanded to colonise a significantly greater proportion of bare ground than the native competitor *Anagallis tenella*, but not than the other two species in the experiment. *Crassula helmsii* did not completely smother or exclude any of the three native species. In a separate field-based experiment, the re-colonisation of *C. helmsii* was monitored, and the speed and extent of re-colonisation was compared between experimental plots which initially had bare ground and plots which had initially been planted with native vegetation. No significant effect of plot treatment was found, and *C. helmsii* did not expand to become dominating, or form monospecific mats. However, it was apparent that *C. helmsii* re-colonised the area from which it had been cleared, right from the start of the experiment.

Finally, field survey data were analysed to investigate whether *C. helmsii* abundance was negatively correlated with *P. australis* abundance, and negatively correlated with variables indicating abundance and diversity in the co-occurring plant community. It was found that *C. helmsii* was negatively correlated with *P. australis* but was not excluded by this taller, dominant species. *Crassula helmsii* was not significantly negatively correlated with the variables of community abundance and diversity, and thus these data could not be taken as evidence that *C. helmsii* can develop dense, monospecific mats by displacing other plant species.

In summary, based on the outcomes of this thesis, a number of factors have been identified which may facilitate the development of dense, dominating mats of *C. helmsii*. With regards to the abiotic environment, conditions which are likely to favour prolific *C. helmsii* growth include freshwater, which is a neutral to alkaline pH, and ground-level conditions where there is little or no overhead shading. An environment with high resource availability in the form of bare ground can promote the spread of *C. helmsii* because this species can adeptly spread vegetatively to take advantage of such resources. Further, *C. helmsii* may do so ahead of other plants where *C. helmsii* is able to suppress the growth of other species, or when the seasonal conditions prevent rapid growth of other species. Implicit in a system with available bare ground, is a lack of co-occurring vegetation. Linked to this, the level of biotic resistance from the resident plant community also appears to be an important factor influencing *C. helmsii* dominance. *Crassula helmsii* may be more likely to form monospecific cover in habitats where native competitors have been removed, and in particular *C. helmsii* may experience a release from competition where tall, canopy forming plants are absent. In this respect the ecology of *C. helmsii* reflects a

wider trend seen in invasive plant ecology; that disturbance events which cause the release of available resources can facilitate non-native invasive plant colonisation (Hobbs & Huenneke, 1992; Burke & Grime, 1996; Davis *et al*. 2000; Pyšek *et al*. 2012a).

Implications: the ecological impacts of C. helmsii

A better understanding of the mechanisms which allow *C. helmsii* to develop dense, dominating vegetative mats, has in turn provided insights into how *C. helmsii* invasion can have a negative impact on ecosystems, by indicating the types of plant species which may be vulnerable to competition from *C. helmsii*. In outline, *C. helmsii* may not have the same level of negative impact on all plant species, and there appear to be a number of wetland plant species which can coexist well with *C. helmsii*, potentially making community-level changes from *C. helmsii* invasion difficult to detect. However, plant species which are restricted to open drawdown zones, and plant species which cannot grow up through mats of *C. helmsii*, may be limited or displaced by the spread of this non-native plant. Furthermore, a change from bare ground to mats of vegetation, could affect animals which live and feed within these areas by altering the physical structure of the habitat.

In this thesis, the abundance of *C. helmsii* in the field was strongly negatively correlated with bare ground cover (chapter seven), a relationship which suggests that this species may have been taking advantage of an available resource (Davis *et al*. 2000). It was also shown experimentally that *C. helmsii* responded to available bare ground with rapid re-colonisation and fast growth (chapter six). It therefore appears that *C. helmsii* is very successful at dispersing to, colonising, and filling empty spaces that occur in wetland habitats. This means that already established, mature plants, especially those which are taller than *C. helmsii*, may not be particularly vulnerable to displacement as a result of the encroachment of *C. helmsii* into a habitat. Where *C. helmsii* is invading an established floral community, rather than signifying a loss of resident species, increases in *C. helmsii* abundance may reflect that it is utilising previously unoccupied space ('space filling'). This space filling hypothesis proposed in this thesis, bears similarity to the 'passenger' model of community change (MacDougal & Turkington, 2005), which describes how a non-native plant can increase in abundance if it is able to fill the space which is not occupied by a native plant (Didham *et al.* 2005; MacDougal & Turkington, 2005). A space filling mechanism may explain why, in the field survey data presented in chapter seven, no negative linear relationship was found between *C. helmsii* abundance and co-occurring species diversity or overall community abundance. The results of chapter seven are not sufficient on their own to confidently conclude that *C. helmsii* invasion is never associated with decreases in plant species diversity, as they are only representative of one specific plant community within the extensive range (as described by Preston & Croft, 1997) that *C. helmsii* occupies within the UK, and therefore it is possible that *C. helmsii* could induce

declines in diversity at another site with a different flora. Interestingly however, the author is aware of only one other published study which has investigated the relationship between *C. helmsii* invasion and declines in biodiversity (Langdon *et al*. 2004), and in concurrence with the present study Langdon *et al*. (2004) found no significant change in plant species richness over a four to seven year period within ponds which had been invaded by *C. helmsii*.

Ricciardi & Cohen (2007) discuss that the non-native species which are considered highly successful invaders, displaying high colonisation success and rapid spread, are not always the non-native invasive species which cause the greatest declines in native species diversity. Accordingly traits such as vegetative dispersal (Kolar & Lodge, 2001), tolerance to abiotic variation (Theoharides & Dukes, 2007), and a fast response to available resources (Daehler, 2003; Davidson *et al*. 2011), may confer invasive success to *C. helmsii* across a large proportion of Western Europe but do not necessarily also allow *C. helmsii* to displace other plants. Houlahan & Findlay (2004) also showed that not all non-native invasive plant species are strongly associated with declines in native plants. The authors studied four wetland plant species of varying morphology, which were non-native to Ontario, Canada, and were known to sometimes achieve community dominance. Of these four potential community dominants, only one species (the graminoid *Phalaris arundinacea*) was found to be significantly negatively associated with native species richness. In contrast however, Stiers *et al*. (2011) found that ponds which were 'semi-invaded' or 'heavily invaded' by one of the three non-native invasive species (*Hydrocotyle ranunculoides*, *Ludwigia grandiflora*, or *Myriophyllum aquaticum*) had significantly lower plant species richness than ponds which were uninvaded, with stronger negative impacts seen on the abundance of submerged and floating species compared to emergent species.

The impacts of non-native invasive plants have been found to be context dependent (Pyšek *et al*. 2012b), controlled in part by the competitive strength of the invader compared to the resident species (Dukes, 2002; Hejda *et al*. 2009). Whilst many of the wetland plant species which were commonly encountered during field work for this research project, such as *Phragmites australis*, *Mentha aquatica*, and *Eleocharis palustris* (appendix III), may be able to tolerate the encroachment of *C. helmsii*, there may nevertheless be particular set of native plant species which are out-competed and therefore negatively impacted by *C. helmsii*. Both Didham *et al.* (2005) and Bauer (2012) discuss a theoretical compromise with regards to the driver-passenger theory of community change, in which an invading non-native species may benefit from declines in the resident community whilst also directly imposing further community change. Therefore in the case of *C. helmsii*, it could be that this species acts mainly as a space-filler, but still directly displaces a small number of plants in the community. Because *C. helmsii* is very successful at colonising bare ground, the plant species which may be vulnerable to competition from *C. helmsii* are likely to be small, low growing plant species which are specialists of open,

bare ground habitats. Examples of such plant species which grow in the UK are; *Baldellia ranunculoides*, *Elatine hexandra*, *Limosella aquatica,* and *Pilularia globulifera* (Hill *et al*. 2004; Abrahams, 2005). These types of plant tend to persist on drawdown zones because disturbance from fluctuating water levels generally limits the survival of more vigorous competitors (Hill *et al*. 2004; Abrahams, 2005), the invasion of *C. helmsii* along a drawdown zone would thus disrupt this dynamic. From site observations made during this research project it is known that *B. ranunculoides*, *P. globulifera*, and *E. hexandra* do occur in habitats which have also been invaded by *C. helmsii*; Castle Water, The New Forest ponds (Chapter two, section 2.1.2.), and Little Sea (chapter six, section 6.4.1) respectively; with this confirmed habitat overlap indicating that competition from *C. helmsii* is a realistic threat to these species. Moreover, out of the three native test species which were grown alongside *C. helmsii* for the common garden experiment in chapter six, it was *Anagallis tenella* which showed to be most greatly limited by competition from the non-native species (section 6.3.), and *A. tenella* is another small plant which grows on open, bare ground patches in wetland habitats (Hill *et al*. 2004). Contrastingly, *Litorella uniflora* is a small plant which grows on bare substrate along wetland margins (Hill *et al*. 2004), but Denton (2013) presents case study evidence to suggest that *L. uniflora* may be able to competitively suppress *C. helmsii*.

Where previously bare ground becomes covered by a mat of vegetation, this could inhibit germination or seedling survival, and therefore larger plant species which nevertheless require bare ground to regenerate could also experience declines due to the spread of *C. helmsii*. Indeed this is also a point made by Langdon *et al*. (2004) who found that a covering of *C. helmsii* can suppress the germination of some pond plant species. From the results of chapter seven (section 7.3.) it was apparent that plant species with a solely sexual reproductive strategy represented only a small proportion of the abundance of the co-occurring plant community, and during the process of collecting trait information it was noticeable that nearly all species were perennials. Therefore, it is possible that sexually reproducing and annually germinating species are suppressed in *C. helmsii* invaded habitats. In contrast, perennial species which spread via rhizomes may be less negatively impacted as the parent plants can generate new peripheral shoots which grow through the *C. helmsii* layer. Plant species which rely on mowing or light grazing management, to maintain an unshaded habitat with a short vegetation structure, may also experience increased competitive pressure with the invasion of *C. helmsii*, as this non-native can attain high abundance in such conditions. This type of interaction was discussed in reference to *Teucrium scordium* in chapter five (section 5.4.).

This thesis focused on investigating the interactions between *C. helmsii* and the co-occurring plant community, and therefore focused on how *C. helmsii* invasion could have a negative impact on plants. However if high *C. helmsii* abundance is most likely to develop in unshaded wetland areas with ample bare ground, it is likely that animal species which utilise such habitat

may be impacted by the development of dense mats of *C. helmsii*. It is hypothesised here that these impacts will arise primarily due to the change in the physical structure of the habitat, from bare ground to vegetated, and may in particular affect wetland invertebrate taxa such as insects, molluscs, and crustaceans. For instance vegetation provides hiding places, for prey species but also for ambush predators, and therefore the growth of *C. helmsii* mats could affect predator-prey interactions. Dense submerged and emergent vegetation in the shallows of water bodies could impede the movement of free-swimming invertebrate taxa, and dense vegetation growing on margins above the water line could affect the microclimate at ground level, for example by preventing direct sunlight reaching the substrate. Some species could benefit from the development of vegetated margins, whereas other species may experience declines, overall causing a shift in the composition of the invertebrate community. Invertebrates which could be especially negatively affected by the loss of bare ground include a number of species of Coleoptera which rely on this particular habitat, for example *Omophron limbatum* which constructs burrows within damp bare sandy substrate (Hodge & Yates, 2000), and also species of Odonata such as *Aeshna cyanea* and *Cordulegaster boltonii* which require bare mud at the edge of water for oviposition (Merritt, 1995; Abrahams, 2005). Changes to the invertebrate community could in turn affect vertebrate species, notably if the availability of invertebrate prey was altered, either through a change in actual population numbers, or a change in how easy the invertebrates were to locate and catch. Wading birds and waterfowl which feed within reedbeds, or in the shallows of water bodies, would be likely to be affected in this way. Birds which glean invertebrates from along bare mud margins; for example members of the *Charadriidae* family (plovers and lapwings); could be particularly negatively affected by *C. helmsii* invasion due to a reduction in suitable feeding habitat.

Implications: the control of C. helmsii

If *C. helmsii* can increase in abundance by filling space, such information has relevance when giving recommendations for its control. In outline, land managers should be vigilant for signs of *C. helmsii* colonisation where bare ground is prevalent in a habitat, and should limit activities which create bare ground and activities which cut or remove tall vegetation. Specifically encouraging native wetland plant species to grow into available bare ground may be a strategy of limiting *C. helmsii* dominance. A problem exists however, when methods which could limit the abundance of *C. helmsii* would also limit the abundance of rare native plant species.

Sites identified as being more vulnerable to the development of dense dominating *C. helmsii* mats should be monitored more intensively for signs of *C. helmsii* colonisation, and where *C. helmsii* has colonised, more resources should be invested into locally eradicating it or maintaining its abundance at a low level. Within the preferred abiotic range of *C. helmsii*; approximately pH 6 – 9 (chapter three) and below 8 ppt salinity (chapter four); wetland nature

reserves which contain large patches of bare ground may be particularly vulnerable to the development of extensive *C. helmsii* cover. An example of this is coastal and floodplain grazing marsh, where fluctuating water levels and trampling by cattle create bare mud around the margins of pools and drainage ditches (Buglife, 2013). Furthermore, newly created wetland habitats such as freshly dug scrapes and ponds or lake systems created on disused aggregate / material extraction sites (Green, 1996), may be especially vulnerable to *C. helmsii* domination, because as well as containing large patches of bare ground, they would also lack mature vegetation or a substantial seed bank (Zedler, 2000) and thus would have even lower biological resistance to non-native invasive spread. Consideration of the characteristics of the flora within a habitat might also provide an indication of its vulnerability to high *C. helmsii* abundance and dominance. In particular, communities where many species do not main year-round above ground vegetation cover might be more vulnerable, as *C. helmsii* could spread and occupy space during periods when it has been vacated by other plants. Examples of this include plant communities with many annual species or species which senesce at the end of the main growing season, and which only regenerate from seed. Also, ephemeral plant communities of drawdown zones which show sequential die off and recolonisation in response to inundation and exposure disturbance from fluctuating water levels (Abrahams, 2005; Bournette & Puijalon, 2010).

Based on the conditions which have been found to encourage high *C. helmsii* abundance, there are certain land management activities which are inadvisable or which should be limited, in sites where *C. helmsii* has invaded or sites near to where *C. helmsii* has invaded. One such activity is the practice of liming acidic ponds, to enhance plant productivity and reduce pH fluctuations (Wurts & Masser, 2013), which is inadvisable because this raises the overall pH of the water. Reducing the acidity and making the pond water more neutral would make abiotic conditions more favourable for *C. helmsii* (chapter three), and indeed Denton (2001) described a case study where *C. helmsii* invaded an acidic pond only when liming had increased the pH. Primarily however, land managers should be cautious in the implementation of activities which remove existing native vegetation and increase the amount of available light and space at ground level, keeping such activities to a minimum. If ground works or vegetation clearance has to be conducted in order to fulfil other management objectives, to avoid facilitating higher *C. helmsii* abundance these should not be carried out in late summer and autumn, when *C. helmsii* can still grow and spread to utilise the available resource but more seasonally constrained competitors cannot. Grazing regimes should be designed in order to ensure that the plant competitors of *C. helmsii* persist within the habitat. For instance it might be best to avoid allowing selective grazers (e.g. sheep) onto exposed drawdown zones in spring and early summer where they could reduce the survival of seedlings and new shoots, and to avoid allowing cattle to remove too much tall vegetation later on in the growing season.

With regards to the removal of *C. helmsii*, land managers should avoid implementing clearance control (biomass removal or degradation) as a stand-alone strategy. Strategies to implement alongside clearance control are discussed in the section below. This recommendation is made because simply killing or clearing the vegetation is unlikely to be a successful strategy for controlling this species long-term; such activities return a site to bare ground, thus creating conditions which are highly suitable for *C. helmsii* colonisation, spread and dominance. As an example of this, *C. helmsii* quickly re-colonised the experimental area at Little Sea (chapter six), arriving within weeks of the black plastic being removed, a finding which concurs with a previous trial of this particular control method (Wilton-Jones, 2005). This also reflects the wider literature, as many studies have reported that after the clearance of a non-native invasive plant, the invader has re-colonised, or another non-native invasive plant has filled the gap (Hulme & Bremner, 2006; Mason & French, 2007; Reid *et al*. 2009; Jäger & Kowarik, 2010). In this way, repeating clearance control over sequential months and years to keep *C. helmsii* re-growth in check is also self defeating, as bare ground conditions are repeatedly being created.

In sites vulnerable to high *C. helmsii* abundance, there are measures which might be valuable to incorporate into land management schemes. In newly created habitats or habitats which naturally contain extensive bare ground patches, the most effective method of preventing dense *C. helmsii* growth would be to stop the plant from colonising in the first place. Indeed it is well acknowledged that the most effective defence against non-native invasive species is a pre-emptive approach (IUCN, 2000; Davis, 2009). National biological security initiatives such as 'Check-Clean-Dry' (GB non-native species secretariat, 2014) and organisation specific recommendations (Day, J. & Gilbert, J. personal communication) provide practical guidelines to prevent the dispersal of viable vegetative propagules, and so will not be discussed in detail here. In sites where *C. helmsii* has already colonised or where a nearby source of colonising propagules makes colonisation hard to prevent, increasing biotic resistance by encouraging the growth of native plants could be a way of limiting the extent to which this species could attain dominance. Similarly (and in reference to the section above) where clearance control of *C. helmsii* has been carried out, encouraging native plants to colonise the cleared areas could be a way of limiting *C. helmsii* re-growth if not a way of totally preventing its re-colonisation. Ensuring a sufficient supply of native propagules to cleared areas has been suggested in the literature as a method of promoting the development of the native species community (Erskine Ogden & Rejmánek, 2005; Reid *et al*. 2009; Stevens & Fehmi, 2011). Thus it could be beneficial to ensure that there are no barriers to the recruitment of native wetland plants in areas cleared of *C. helmsii*. Furthermore, native species, especially species which are known to produce vigorous horizontal growth across bare ground (such as *Hypericum elodes*, chapter six), could be deliberately planted or seeded in *C. helmsii* cleared areas. Land managers could also plant a flora which is tolerant to the conditions of stress and disturbance commonly experienced within a particular habitat therefore limiting the amount to which native vegetation might

experience die back. For example planting native species which are tolerant to sequential inundation and exposure in seasonal wetland pools and drawdown zones, encouraging a ground level flora which is adapted to grazing and trampling where livestock are used to maintain an open vegetation structure, or planting species which can survive salinity of 8 ppt or greater where brackish water inundation is to be used. Of course this selection for tolerant species should happen naturally in habitats, but this process could be prevented if *C. helmsii* were to colonise and spread before more tolerant native species could become established. As presented in chapter four, *C. helmsii* could be killed with water of above 8 ppt salinity. If it is decided to implement this strategy to eradicate *C. helmsii* from an area, firstly it is important to ensure that all *C. helmsii* vegetation is totally submerged, because *C. helmsii* can 'escape' from this treatment if it is able to grow up the margins and away from the water (Money, S. personal communication). Secondly, to achieve long term elimination of *C. helmsii* a site would need to be maintained above 8 ppt salinity. Post-treatment sites with natural freshwater inputs (e.g. Charlton *et al.* 2010) would likely become less saline over time, and it is probable that if salinity levels at a site dropped back into the tolerance range for *C. helmsii*, that site would again be vulnerable to re-invasion. Maintenance at above 8 ppt could perhaps be achieved by constructive a permanent connection to a source of saline water. For example coastal lagoons could be connected to sea water via drainage ditches (Yates, B. personal communication).

Recommendations such as planting vegetation on bare ground to limit *C. helmsii* dominance, or allowing taller vegetation to persist, may conflict with other management objectives in some cases. For example, because of the species assemblages associated with bare ground drawdown zones, which include rare species of plants and animals (Hodge & Yates, 2000; Abrahams, 2005), some wetland areas are managed specifically to provide exposed mud (Williams *et al*. 2007). Therefore, it is not a viable option to increase biotic resistance by deliberately planting vegetation in such habitats. Similarly, habitats are often specifically managed to include areas of vegetation with an 'open' structure, as this enhances beta diversity by allowing species to persist which would not grow under a taller closed canopy. Indeed fens are managed to limit the dominance in places of tall plants such as *Epilobium hirsutum* and *Phragmites australis* (McBride *et al*. 2011), species found in this thesis to compete strongly against *C. helmsii* (chapters five and seven). Thus by encouraging the persistence of taller vegetation to suppress *C. helmsii,* other native species might also be suppressed. An example of this was shown in chapter five; the abundance of *C. helmsii* declined in the grazing exclosures, attributed to competition from *E. hirsutum*, however the abundance and frequency of *Teucrium scordium*, a nationally rare plant that requires an open vegetation structure (Beecroft *et al.* 2007), was also lower in the grazing exclosures. Perhaps one of the biggest impacts of *C. helmsii* is that it can 'hijack' implemented conditions in managed habitats, so that standard methods of increasing biodiversity are no longer effective, and just serve to facilitate *C. helmsii* spread.

Limitations and further work

The strategy taken in this thesis was to conduct a number of different experiments and surveys, covering different questions regarding the ecology of *C. helmsii*. The rationale behind this strategy being that overall, more could be learned about this non-native invasive plant species. This meant however, that the time which could be spent on each experiment or survey was restricted by the need to collect data for other objectives, within the time frame allocated for the research project. The strategy was successful in that reference could be made to abiotic, plant-plant, and plant-herbivore interactions within the main conclusion of the discussion. However the alternative strategy, of allocating research time to a smaller number of objectives and research questions, may have provided stronger individual conclusions by allowing for additional replication. Firstly, in the common garden experiment (chapter six) it could be seen that there was between-replicate variation within each of the treatment groups, which in some groups, may have influenced the outcome of statistical analysis. Thus this experiment may have benefitted from a sample size of greater than five per treatment group. As it was, the method of taking photographs of each replicate every four weeks (490 photographs taken overall) and then digitally counting the grid cells for all of these photographs (an average of 1151 grid cells per photograph) was a time consuming activity. Including more replicate plots would have considerably increased the time spent on this experiment. Secondly, the field experiments presented in chapters five and six (field experiment) were each carried out in just one location. If it had been possible to have replicated these experiments in locations with different abiotic conditions and species assemblages, this would have additionally provided an indication of the generality of the interactions seen between *C. helmsii*, grazing animals, and the plant community. Thirdly, it is acknowledged that abiotic conditions and plant abundance vary seasonally in wetland habitats (Brönmark & Hansson, 1998). It would have been beneficial to have conducted the field survey (chapters three and seven) across three or four seasons to determine whether the same statistically significant relationships persisted.

With additional time, the research presented here could have been expanded to investigate the research questions in more depth, and additional research questions could have been added, to understand more about the ecology of *C. helmsii*. Three potential avenues of further study are discussed here. Firstly, an important area of additional research would be to disentangle the relationship between *C. helmsii* abundance, the amount of overhead shading, and the abundance of co-occurring tall plant species. It was found that *C. helmsii* abundance was negatively correlated with shade (chapter three), negatively correlated with *P. australis* (chapter seven), and showed a decline in abundance as *E. hirsutum* increased in abundance (chapter five). The hypothesis discussed above was that tall vegetation suppresses *C. helmsii* abundance due to a shading effect. However, it cannot be implicitly assumed that these statistically significant relationships are causal, and furthermore, *C. helmsii* abundance was not significantly correlated

with the abundance of plants in the trait categories 'tall' or 'v.tall' (from which *P. australis* was excluded) (chapter seven). It needs to be experimentally ascertained, that there is indeed direct competition between *C. helmsii* and these tall, competitive species. If causality is demonstrated, and it is found that *C. helmsii* abundance can be suppressed by competition, the next stage would be to investigate what factors are involved in this relationship. As discussed *C. helmsii* could experience light limitation, but it could also experience nutrient limitation, or simply have less space available for expansion. Mesocosm growth experiments would be an appropriate method of investigating competition between *C. helmsii* and tall competitive wetland plant species; species can be planted at varying ratios to investigate the strength of suppression (how much of a size asymmetry is required for suppression to be evident) (e.g. Meyer *et al*. 2010; Leger *et al*. 2014), and also allow abiotic factors of interest such as shade and nutrient levels to be manipulated, whilst controlling for other confounding factors (e.g. Abraham *et al*. 2009; Xie *et al*. 2013).

Secondly, invasion success is more likely in an area to which a large number of propagules have been dispersed, or to which propagules frequently disperse (Lockwood *et al*. 2005; Simberloff, 2009a). Furthermore, a high propagule pressure may aid a non-native species in persisting despite sub-optimal abiotic conditions (D'Antonio *et al*. 2001). Thus propagule pressure may have been an additional significant explanatory variable, related to *C. helmsii* abundance, in the field survey dataset (chapters three and seven). Further work, which incorporated propagule pressure as an additional explanatory variable, would need to acknowledge that it is not of interest per se whether the propagule pressure affects colonisation success, but instead whether the propagule pressure at colonisation, has any influence on how dominant *C. helmsii* later becomes in the plant community. Instead of a field survey it might be more efficacious to conduct a controlled field experiment (e.g. von Holle & Simberloff, 2005), as this would allow propagule pressure to be manipulated to produce treatments with varying numbers of propagules.

Thirdly, it is possible that disturbance related to changing water levels may have been an additional important factor influencing the experimental outcomes, as has been recorded in other invaded wetland systems (Schooler *et al*. 2010; Price *et al*. 2011). In particular, both the grazing exclosure experiment (chapter five) and the bare ground / vegetated plot field experiment (chapter six) were located on lake margins which were seen to be varyingly flooded or exposed. It is not known to what extent the abundance of *C. helmsii* was independently affected by the inundation regime in these experiments, or whether the inundation regime influenced interspecific competition between *C. helmsii* and the resident native species. Growth experiments, which manipulated inundation in a controlled manner, would be an effective method of ascertaining how *C. helmsii* responds to this type of disturbance, and how *C. helmsii* responds relative to selected native competitors.

Additional research time could have been used to conduct more work to understand how dominating *C. helmsii* growth has an impact on native plant species. For instance, there could have been a greater focus on testing whether *C. helmsii* has a more negative impact on plant species with particular traits. The traits of plants species were considered in the analysis for chapter seven, and due to the low representation of plants with an annual life cycle and sexual reproduction, it might be informative to specifically test whether *C. helmsii* outcompetes these types of plants more so than perennials which can spread clonally. If more field data were to be collected for this objective, it would be important to first consider how many annual seeding species (compared to perennial clonal species) might be expected to be found within a habitat irrespective of the presence of *C. helmsii*. Specialists of bare ground drawdown zones have been highlighted in this discussion as species which might be particularly negatively affected by *C. helmsii*. This hypothesis could be tested in a number of ways, for instance surveys of *C. helmsii* invaded and non-invaded drawdown zones within the same habitats could be used to determine whether the native species of interest had a lower abundance or were absent where *C. helmsii* occurred. Similarly, time series data of *C. helmsii* colonising a bare ground drawdown zone could be analysed for declines in key native plant species. Common garden experiments could also be used to compare the competitive ability of *C. helmsii* to that of the native drawdown zone species in question, similarly to the experiment presented here in chapter six (section 6.2). It might also be valuable to conduct further research which tests how well *C. helmsii* competes against other non-native invasive plants of wetlands, such as *Myriophyllum aquaticum* and *Hydrocotyle ranunculoides*, and further whether a combination of non-native invasive plant species, for example *C. helmsii* and *M. aquaticum* growing as emergent plants within a pond (personal observation), has a more negative impact on native flora due to an additive or synergistic effect (Kuebbing *et al*. 2013).

It would be valuable to also direct future research towards testing the impact of dominating *C. helmsii* growth on animal taxa. As with the plants, it may be most pressing to investigate whether *C. helmsii* negatively affects taxa which are specialists of bare ground drawdown zones, thus testing the hypothesis outlined in this discussion that *C. helmsii* has a greater negative impact on species of this habitat type. Such work could include sampling sites for invertebrates or signs of invertebrate habitation (e.g. burrows), and observation of bird feeding behaviour to see if it is altered where *C. helmsii* occurs instead of bare ground. It might also be interesting to investigate whether the appearance of dense vegetation affects the feeding behaviour of invertebrates. For example it could be asked whether the presence of *C. helmsii* flowering on land in late summer could have an impact on the foraging choices made by nectar feeding insects; whilst conducting fieldwork for this thesis it was apparent that the dense mats of flowering *C. helmsii* produced a strong honey-like scent, and that insects such as hoverflies were moving amongst these flowering mats (appendix VI), potentially attracted to a source of nectar. Within the water, it could be asked how aquatic crustaceans such as *Gammarus*

aquaticus, which are non-specialised consumers of plant and animal matter (Kelly *et al*. 2002), might be affected by an additional source of vegetation both as a substrate in which to live and as a food source. To test this, laboratory tank experiments could be conducted to investigate whether similar population sizes of *G. aquaticus* can be sustained in *C. helmsii* stocked tanks, compared to tanks stocked with common native aquatic plants. *Crassula helmsii* does not experience much vegetative die back because it is a hardy evergreen plant (Preston & Croft, 1997; CAPM, 2004), and can grow during the autumn (chapter six) when many other plants are senescing and releasing nutrients. Future research into how the invasion of *C. helmsii* could have an impact on nutrient cycling might therefore also be an informative topic. A sensible hypothesis to test might be; whether the prevalence of available nutrients in aquatic systems during spring, is lowered by the invasion of *C. helmsii*, because this species can assimilate nutrients made available from senescence during the autumn.

In summary this thesis presents data on the ecology *C. helmsii*, which leads on to deeper investigation of the specific situations in which this plant might have a negative impact on invaded ecosystems. Conclusions regarding *C. helmsii* ecology and impacts can also be used in the design of future management strategies to more successfully limit *C. helmsii* growth and dominance. Single-species studies, such as this one, furthermore represent a useful contribution to the literature of non-native invasive plant ecology, acting as case studies which can be used in larger syntheses to confirm or reject the existence of general patterns.

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10. Appendices

I: Data volunteered from *C. helmsii* invaded sites

Table I-1 (and next page). Details of the 36 additional *Crassula helmsii* invaded sites from which data were provided on pH, macronutrient levels, and *C. helmsii* abundance. These data were used in Chapter three to compare against collected field survey data.

Reserve	No. sites	Land owned / managed by	Location	Contributors
Bowdown woods	1	Berkshire, Buckinghamshire, and Oxfordshire Wildlife Trust (BBOWT)	Crookham, Berks	Phil Dean
Greenham Common	2	BBOWT	Crookham, Berks	Phil Dean
Kintbury	1	BBOWT	Kintbury, Berks	Andy Coulson-Phillips
Berryhill Fields	1	Stoke-on-Trent City Council	Stoke-on-Trent, Staffs	Amy-Jayne Dutton
Farrer Hall	2	National Trust	Eastbourne, E. Sussex	Dave Morgan
Filcombe Farm	2	National Trust	Morcombelake, Dorset	John Sibthorpe
Glastry ponds	1	National Trust	Ballyhalbert, County Down	Andrew Upton
Mottisfont	1	National Trust	Romsey, Hants	Rob West
Manor Farm	7	Private land owner	Norfolk	Sayer *et al.* (2012)
				Sayer *et al.* (2013)
Park Hall	2	Stoke-on-Trent City Council	Stoke-on-Trent, Staffs	Amy-Jayne Dutton
Pett Level	1	The Wetland Trust	Pett, E. Sussex	Barry Yates
Arne	1	The Royal Society for the Protection of Birds (RSPB)	Wareham, Dorset	Toby Branston
Blean Woods	1	RSPB	Canterbury, Kent	Michael Walter
Conwy	3	RSPB	Conwy	Sarah Money

Table I-1. Continued.

Reserve	No. sites	Land owned / managed by	Location	Contributors
Dearne Valley	1	RSPB	Barnsley, S. Yorkshire	Charlotte Bell
Dee Estuary	1	RSPB	Neston, Cheshire	Rhian Pierce
Dungeness	1	RSPB	Lydd, Kent	Natalie Holt
Lochwinnoch	1	RSPB	Lochwinnoch, Renfrewshire	Paula Baker
North Kent Marshes	1	RSPB	Rochester, Kent	Cath Dewhurst
Otmoor	1	RSPB	Beckley, Oxford	Martin Randall
Pulborough Brooks	1	RSPB	Pulborough, W. Sussex	Peter Hughes
Rye Meads	1	RSPB	Stanstead Abbotts, Herts.	Vicky Buckel
Sandwell	1	RSPB	West Bromwich, W. Midlands	Leanne Harris
Woolmer Forest	2	South Downs National Park Authority	Hants. / W. Sussex	Katherine Stearne

II: Is there an effect of pH on the growth rate of *C. helmsii*?

An experiment was conducted, which was designed to measure the growth rate of *C. helmsii* in experimentally controlled treatments of pH, at three levels of nutrient concentration.

Methods and materials

To set up the experiment, 27 plastic storage containers (5 L) were used as individual replicates. These were lined with horticultural sand to 3 cm depth and filled with 1 L of water, to mimic shallow water habitat conditions. Containers were placed in a randomised treatment arrangement, in an outdoor location at Bournemouth University in Dorset (OS grid ref: SZ 073 937). Translucent lids were placed on the plastic containers, to shield from rain and detritus, which were propped open to allow for transpiration and prevent overheating.

Three levels of pH were used; pH 5.0, 7.0, and 9.0. These were combined in a factorial design with three levels of nutrient concentration; 0.5, 0.25 and 0.125 x full strength modified Hoagland solution (Taiz & Zeiger 2006). In total there were 9 different treatments, with 3 replicates per treatment. Treatments were applied as a solution, in the 1 L water added to each replicate. The treatment levels were set by adding the correct concentration of nutrient solution, and then adjusting the pH by adding 0.1M HCl or NaOH, to lower or raise the pH of the solution respectively. Care was taken to adjust the pH slowly, minimising the chances of over shooting the required pH and thus having to add additional acid or alkali, as it was unknown whether these compounds would have an effect on plant nutrition. For this reason, it was decided to avoid also adding buffer solutions to further stabilise the pH of the nutrient solutions.

The experiment was run over 26 days between 12th August and 7th October 2011. At the start of the experiment each replicate was filled with the correct 1 L pH adjusted nutrient solution, and then 10 g (± 0.01 g) fresh weight of *C. helmsii* was added to each replicate. Distilled water which had been pH adjusted was added subsequently, every week throughout the duration of the experiment, to maintain the correct pH treatment levels. The pH of the water within each replicate was recorded prior to the addition of fresh solution in order to ascertain the amount of change in pH experienced within the treatment mesocosms. At the end of the experiment, the *C. helmsii* from each replicate was separately rinsed thoroughly to remove attached sand and algae, and dried at 90˚C for 48 hours, before being weighed to obtain an end dry weight. A start dry weight value was estimated by weighing out twenty additional *C. helmsii* samples of 10 g fresh weight, drying them at 90˚C for 48 hours and taking the mean dry weight of these samples.

The relative growth rate of *C. helmsii* was calculated for each replicate by using the formula:

$$\text{Relative growth rate} = \ln(W2) - \ln(W1) / (t2 - t1)$$

Where W1 is the start and W2 the end dry weight, ln is the natural logarithm, and t1 is the start and t2 the end time measured in days. The formula describes the proportional increase of a plant in grams, expressed per gram per day ($g\ g^{-1}/day^{-1}$) (Hunt, 2003).

Results

The experiment was carried through to completion, and results were obtained for the relative growth rate of *C. helmsii* in each pH / nutrient concentration treatment (Fig. II-1). However, the results for the effect of pH were considered unreliable, because there was too much change in the pH during the course of the experiment. In most replicates the pH had moved towards to neutral to alkaline pH by the end of each week (Table II-1), and so between treatment differences could not be confidently attributed to differences in pH treatment level.

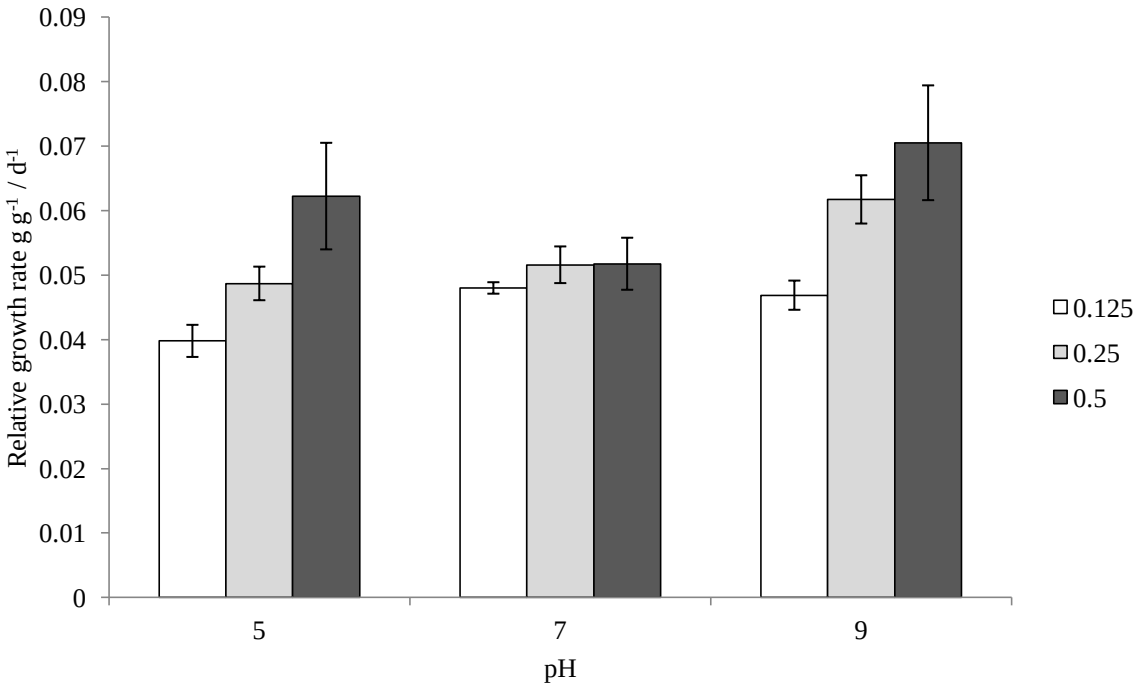

Figure II-1. Bar charts showing the relative growth rate (± 1 SE) of *Crassula helmsii* when grown in a factorial design combining three treatment levels of pH (5.0, 7.0, and 9.0), and three treatment levels of nutrient dilution (0.5, 0.25, and 0.125 x full strength nutrient solution).

Table II-1. The mean pH of the water measured in each treatment, at the end of each week of the experiment. Also indicated is the amount of change in pH away from the required treatment level, and whether the pH was too high (↑) or too low (↓).

Treatment		End week 1			End week 2			End week 3			End week 4		
pH	Nutrients	pH	Change		pH	Change		pH	Change		pH	Change	
5.0	0.125	7.4	↑	2.4	8.4	↑	3.4	9.0	↑	4.0	8.6	↑	3.6
5.0	0.250	6.2	↑	1.2	8.5	↑	3.5	9.0	↑	4.0	8.9	↑	3.9
5.0	0.500	5.6	↑	0.6	7.9	↑	2.9	8.7	↑	3.7	8.8	↑	3.8
7.0	0.125	7.8	↑	0.8	8.7	↑	1.7	9.3	↑	2.3	9.3	↑	2.3
7.0	0.250	7.3	↑	0.3	8.7	↑	1.7	9.5	↑	2.5	9.5	↑	2.5
7.0	0.500	7.0	−	0.0	8.6	↑	1.6	8.7	↑	1.7	9.1	↑	2.1
9.0	0.125	8.5	↓	-0.5	9.7	↑	0.7	9.7	↑	0.7	9.9	↑	0.9
9.0	0.250	8.2	↓	-0.8	10.1	↑	1.1	10.0	↑	1.0	9.6	↑	0.6
9.0	0.500	8.5	↓	-0.5	9.0	−	0.0	8.9	↓	-0.1	9.1	↑	0.1

III: Plant trait matrix

Table III-1. The list of trait categories included within the trait matrix. These were used to categorise the plant species which were recorded in the survey detailed in Chapter seven.

Category	Trait	Interpretation	Data sources
Main reproductive strategy	sexual	Sexual reproduction	[1] [2]
	clonal	Clonal reproduction	
	clo.sex	Clonal and sexual reproduction	
Primary clonal organs	fragments	Vegetative fragments	[1] [2]
	rhizomes	Rhizomes (below ground)	
	stolons	Stolons (above ground)	
Leaf persistence	aestival	Leaves are aestival (deciduous)	[1] [2]
	semi_ev	Leaves are semi-evergreen	
	evergreen	Leaves are evergreen	
Typical max height	v.tall	Greater than 100 cm	[1] [2] [4]
	tall	51 to 100 cm	
	medium	26 to 50 cm	
	short	25 cm or less	
	floating	Assumes height of substrate	
Typical stem position	erect	Erect stems	[3]
	climbing	Stems climbing up other plants	
	prostrate	Prostrate stems	
	floating	Stems floating / supported by water	
Typical leaf area	v.small	0.1 – 1 cm^2	[1] [3] [5]
	small	1 – 10 cm^2	
	medium	10 – 100 cm^2	
	large	100 – 1000 cm^2	
	none	No leaves	
Leaf dimensions	narrow	Leaves more than 3 times long as wide	[1] [5]
	broad	Leaves between 1 and 3 times long as wide	
	equal	Leaves equally long as wide	
	none	No leaves	

[1] Fitter & Peat (1994), [2] Hill *et al*. 2004, [3] Kleyer *et al*. (2008), [4] Rose (2006), [5] Rose (1989).

Table III-2. (and next two pages). A trait matrix listing the specific traits of each plant species which was recorded within the field survey of *Phragmites australis* dominated habitat, detailed in Chapter seven.

Species	Trait category						
	Reproductive strategy	Clonal organs	Leaf persistence	Max height	Stem position	Leaf area	Leaf dimensions
Crassula helmsii	clonal	fragments	evergreen	short	prostrate	v.small	narrow
Phragmites australis	clonal	rhizomes	aestival	v.tall	erect	medium	narrow
Agrostis stolonifera	clo.sex	stolons	evergreen	medium	erect	small	narrow
Alisma lanceolata	sexual	none	aestival	tall	erect	medium	broad
Alisma plantago-aquatica	sexual	none	aestival	tall	erect	large	broad
Apium nodiflorum	clonal	fragments	aestival	tall	erect	medium	broad
Calystegia sepium	clonal	rhizomes	aestival	v.tall	climbing	medium	broad
Carex acutiformis	clonal	rhizomes	semi_ev	v.tall	erect	medium	narrow
Carex flacca	clonal	rhizomes	evergreen	medium	erect	small	narrow
Carex riparia	clo.sex	rhizomes	evergreen	v.tall	erect	medium	narrow
Cirsium palustre	sexual	none	evergreen	v.tall	erect	medium	narrow
Eleocharis palustris	clo.sex	rhizomes	aestival	tall	erect	none	none
Elodea nuttallii	clonal	fragments	evergreen	floating	floating	v.small	narrow
Epilobium hirsutum	clo.sex	rhizomes	semi_ev	v.tall	erect	medium	narrow
Epilobium tetragonum	clo.sex	none	semi_ev	tall	erect	small	narrow
Galium palustre	clo.sex	rhizomes	semi_ev	tall	erect	v.small	narrow
Juncus acutiflorus	clo.sex	rhizomes	aestival	tall	erect	small	narrow

Table III-2. (continued).

Species	Trait category						
	Reproductive strategy	Clonal organs	Leaf persistence	Max height	Stem position	Leaf area	Leaf dimensions
Juncus articulatus	clo.sex	rhizomes	evergreen	tall	erect	small	narrow
Juncus bufonius	sexual	none	aestival	short	erect	v.small	narrow
Juncus effusus	clo.sex	stolons	evergreen	v.tall	erect	medium	narrow
Juncus subnodulosus	clo.sex	rhizomes		v.tall	erect	medium	narrow
Lemna minor	clonal	fragments	semi_ev	floating	floating	v.small	broad
Lemna trisulca	clonal	fragments	semi_ev	floating	floating	v.small	broad
Lycopus europaeus	clo.sex	rhizomes	aestival	tall	erect	medium	broad
Lysimachia nummularia	clonal	stolons	evergreen	short	prostrate	small	broad
Lythrum salicaria	sexual	none	aestival	v.tall	erect	small	broad
Mentha aquatica	clo.sex	rhizomes	evergreen	tall	erect	medium	broad
Myosotis scorpioides	clo.sex	stolons	evergreen	tall	erect	small	broad
Persicaria amphibia	clo.sex	rhizomes	aestival	tall	floating	medium	broad
Potentilla anserina	clo.sex	stolons	aestival	short	prostrate	medium	narrow
Ranunculus omiophyllus	sexual	stolons		short	floating	small	equal
Rorippa nasturtium-aquaticum	clo.sex	stolons	evergreen	medium	prostrate	medium	broad
Rumex hydrolapathum	sexual	none	semi_ev	v.tall	erect	large	broad
Samolus valerandi	sexual	none	aestival	medium	erect	small	broad
Senecio aquaticus	sexual	none	evergreen	tall	erect	medium	broad

Table III-2. (continued).

Species	Trait category						
	Reproductive strategy	Clonal organs	Leaf persistence	Max height	Stem position	Leaf area	Leaf dimensions
Solanum dulcamara	clo.sex	stolons	aestival	v.tall	climbing	medium	broad
Stachys palustris	clo.sex	rhizomes	aestival	tall	erect	medium	broad
Teucrium scordium	clonal	rhizomes		tall	erect	small	broad
Typha angustifolia	clo.sex	rhizomes	aestival	v.tall	erect	medium	narrow
Typha latifolia	clo.sex	rhizomes	aestival	v.tall	erect	large	narrow
Urtica dioica	clo.sex	rhizomes	semi_ev	v.tall	erect	medium	broad
Veronica catenata	sexual	stolons	aestival	medium	erect	small	narrow

IV: Insect visitors

Figure IV-1. Observations whilst conducting fieldwork suggest that insects may be attracted to dense flowering mats of *Crassula helmsii*.